

## プレニル鎖延長酵素の構造と機能

著者	古山 種俊
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最終講義

3/6/2009

# プレニル鎖延長酵素の構造と機能

東北大学多元物質科学研究所

古山 種俊



## 略 歴

1945年7月15日

1964年3月 静岡県立下田北高校 卒業

1968年3月 東北大学 理学部 化学科 卒業(田宮信雄教授)

1970年3月 東北大学大学院理学研究科化学専攻修士課程修了(瀬戸秀一教授)

1973年3月 東北大学大学院理学研究科化学専攻博士課程修了・理学博士  
(瀬戸秀一教授)

1973年6月～1974年8月 米国 スタンフォード大学化学科博士研究員  
(E.E.van Tamelen 教授)

1974年9月～1991年3月 東北大学 非水溶液化学研究所 助手  
(瀬戸秀一教授:小倉 協三 教授)

1991年4月～1994年9月 東北大学 反応化学研究所 助手(小倉 協三 教授)

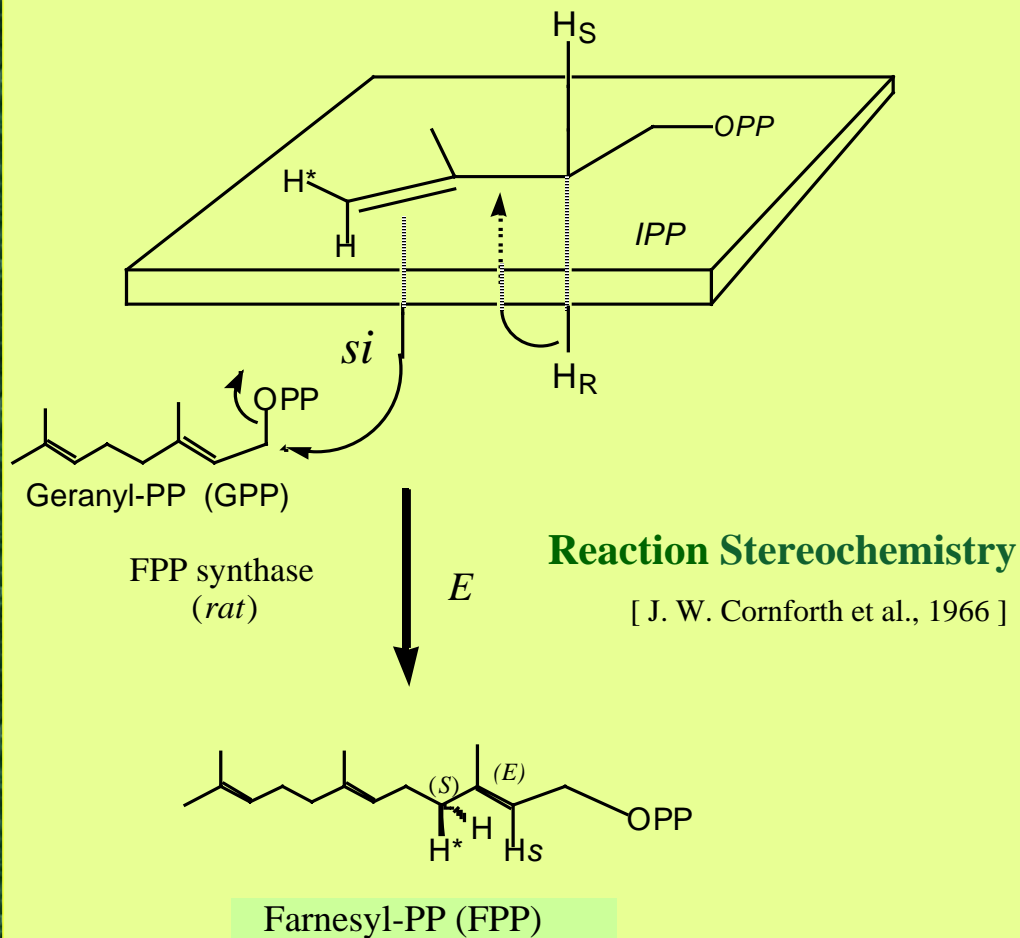
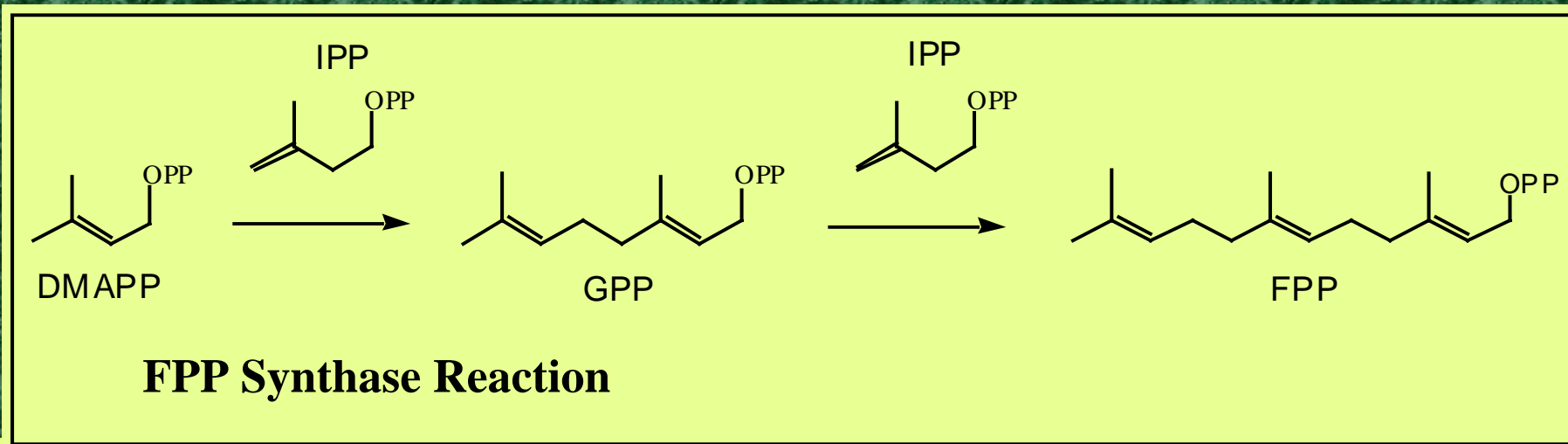
1994年10月～1997年4月 東北大学 工学部生物化学工学科 助教授(西野 徳三教授)

1997年5月～2001年3月 東北大学 反応化学研究所 教授

2001年4月 東北大学 多元物質科学研究所 教授(組織改組)

2008年3月 定年退職

# プレニル鎖延長酵素 (プレニルトランスフェラーゼ) 反応



## 立体特異的炭素 - 炭素結合形成反応

### ○ 人工基質に対する意外に広い基質特異性

Ogura et al. (1970); Nishino et al., (1971); Koyama, et al., (1973) ...

### ○ 厳密な立体特異性

Ogura et al. (1974); Koyama et al. (1977); Kobayashi et al. (1985) ...

### ○ 昆虫幼若ホルモン類の骨格合成

JH I, JH II, JH III, JH 0, 4-MeJH I

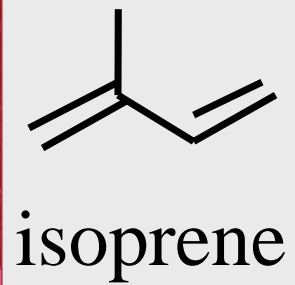
Koyama et al. (1973); (1981); (1985); ...

### ○ 昆虫フェロモンの不斉合成 Faranal

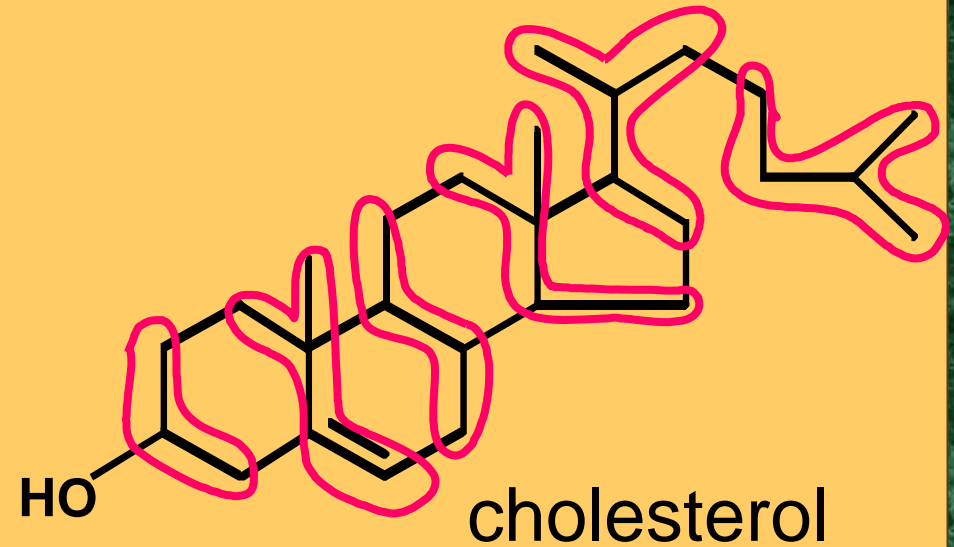
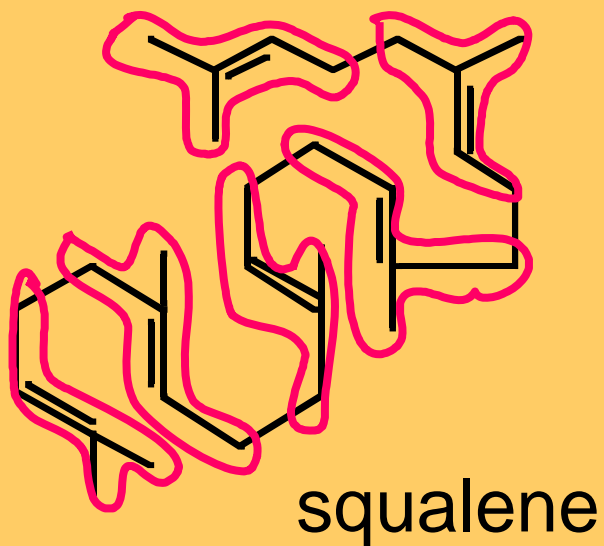
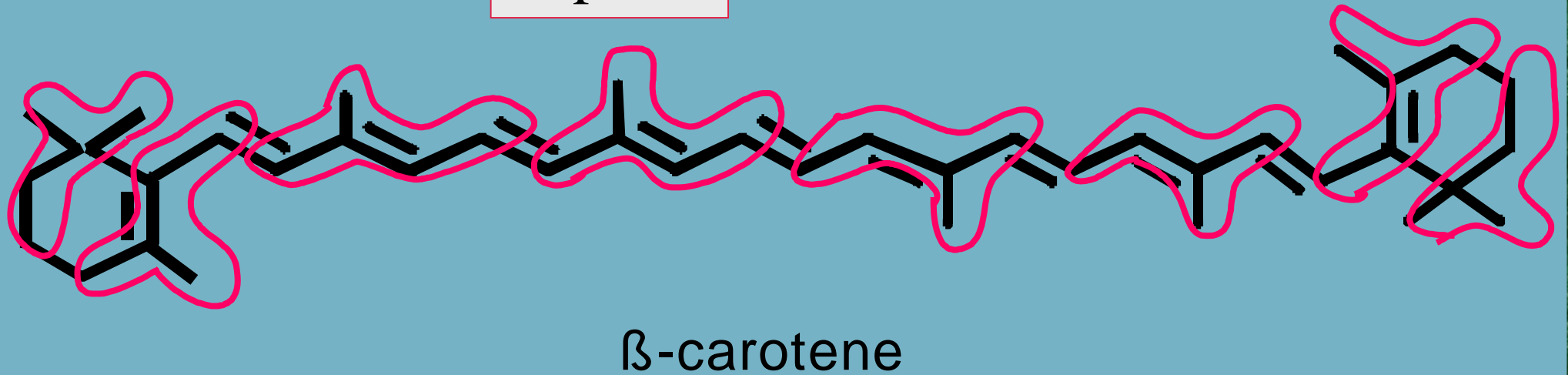
Kobayashi et al., (1980); Koyama et al., (1983); ....



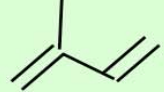
イソプレノイド:



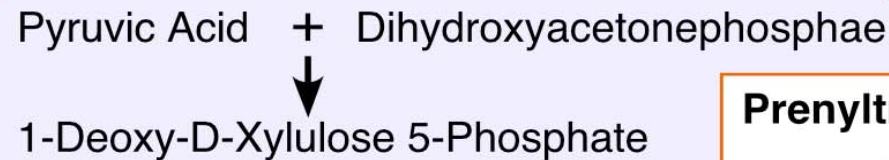
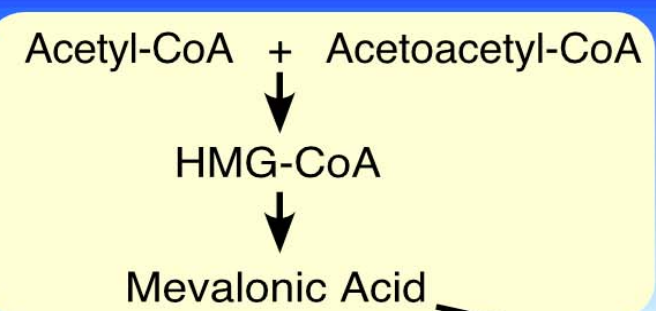
イソプレン ( $C_5H_8$ ) を構成単位とする  
天然有機化合物



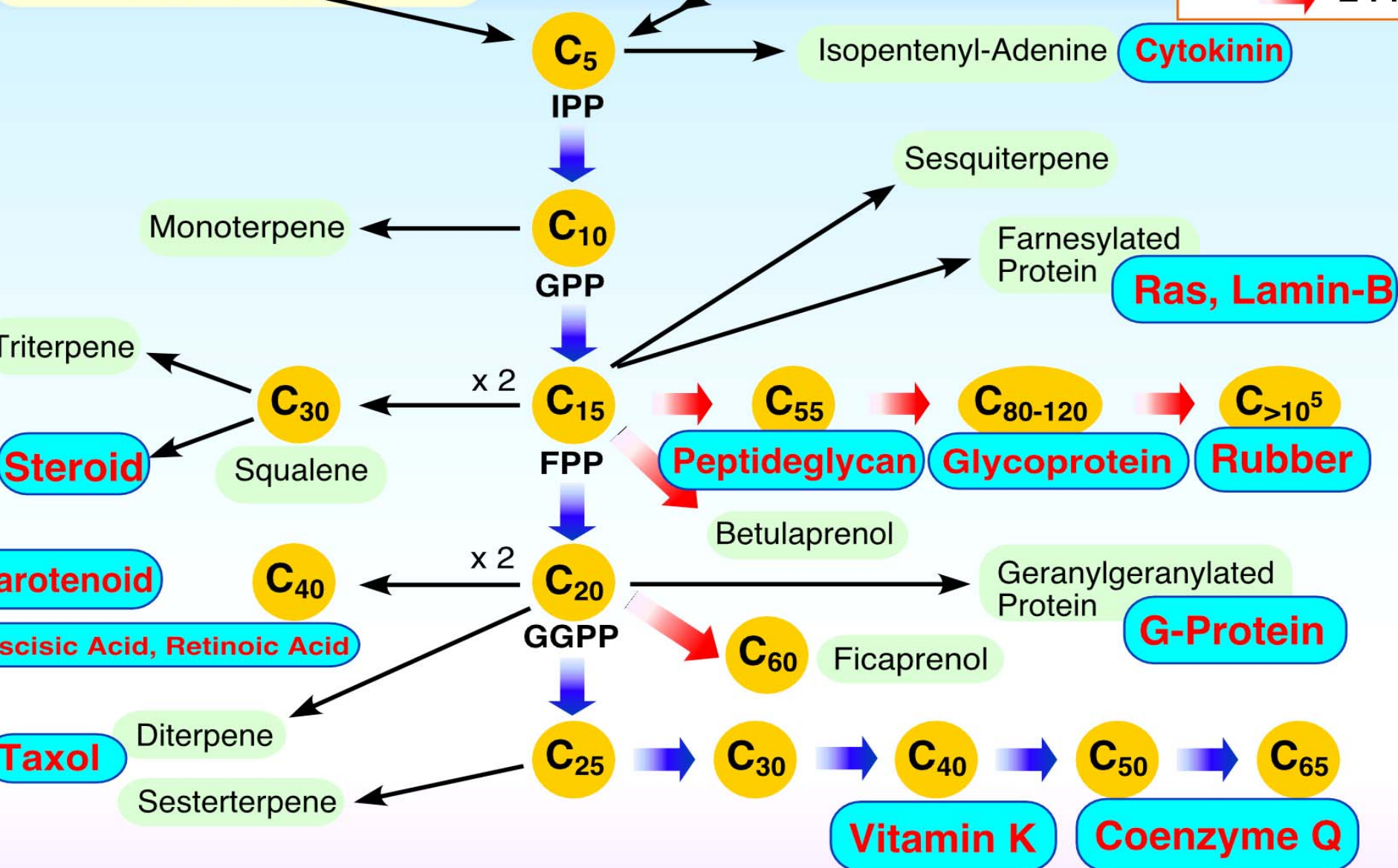
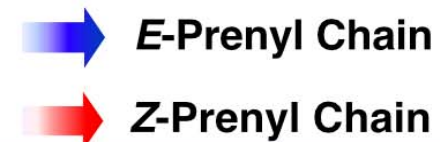
# Isoprenoid Biosynthesis



Isoprene Unit (C<sub>5</sub>)

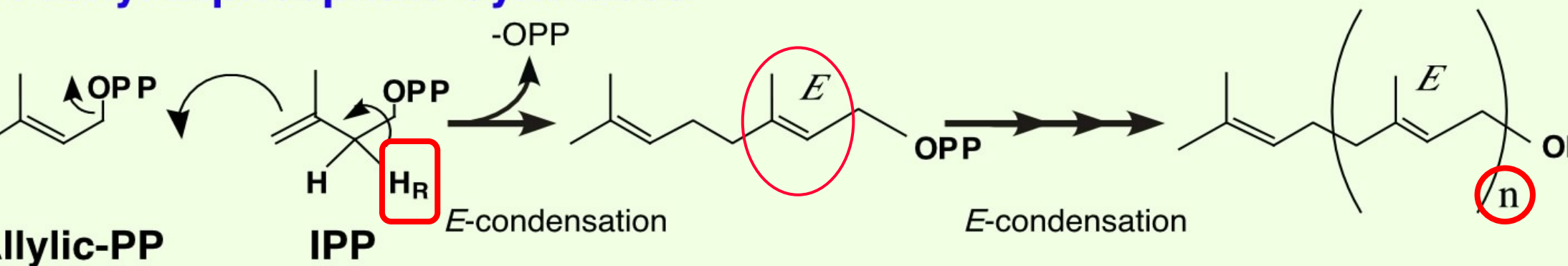


Prenyltransferase Reaction

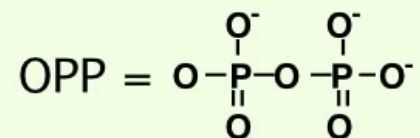
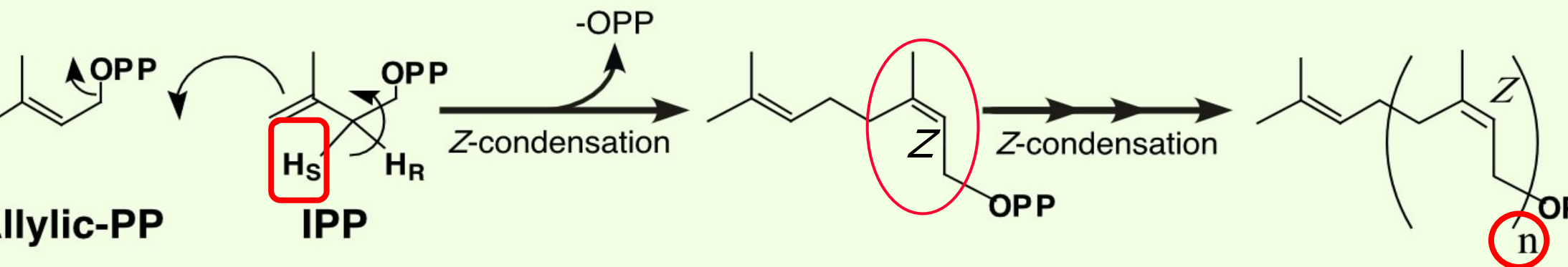


# Enzymatic Prenyl Chain Elongation

## -Prenyl Diphosphate Synthases

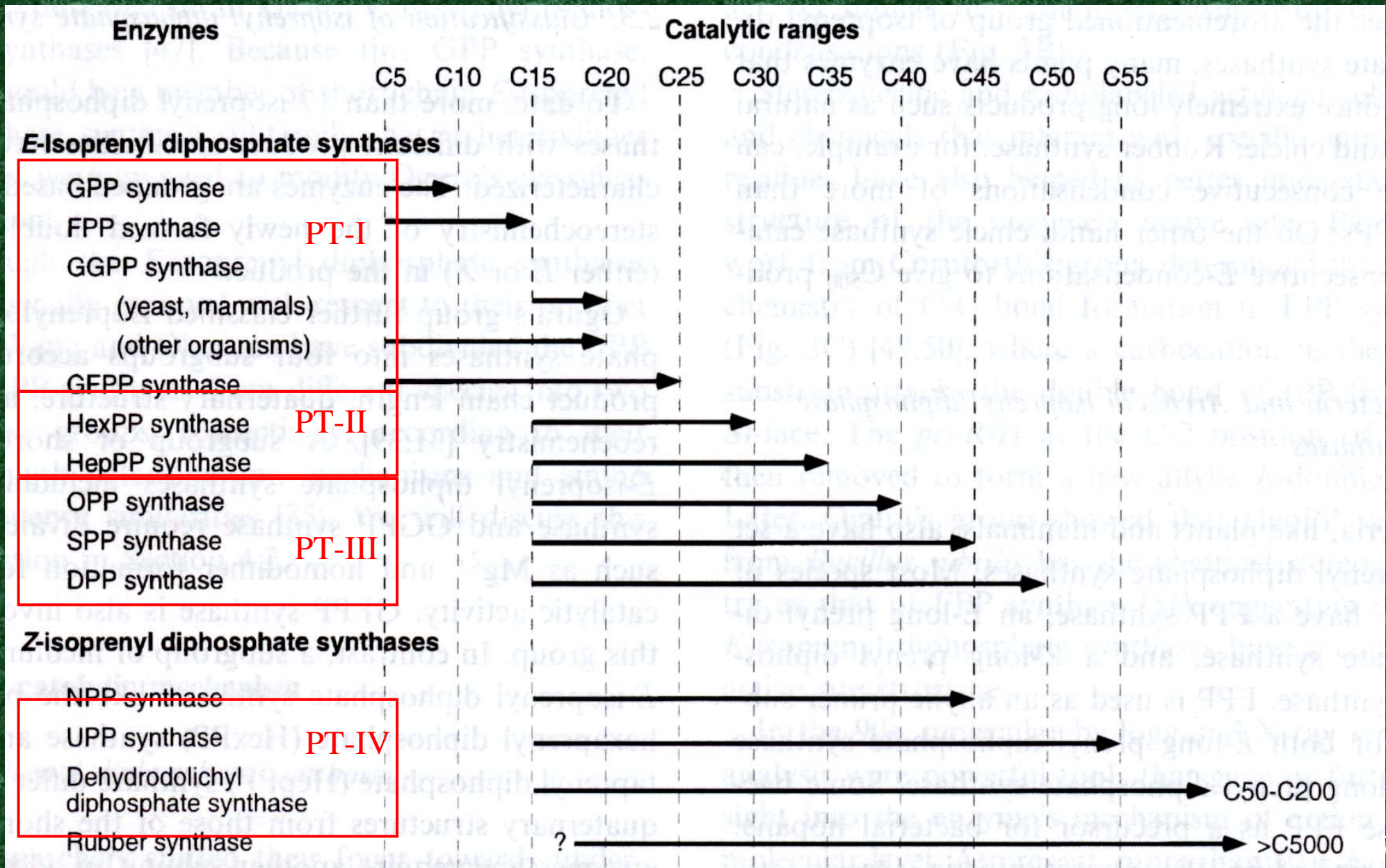


## -Prenyl Diphosphate Synthases

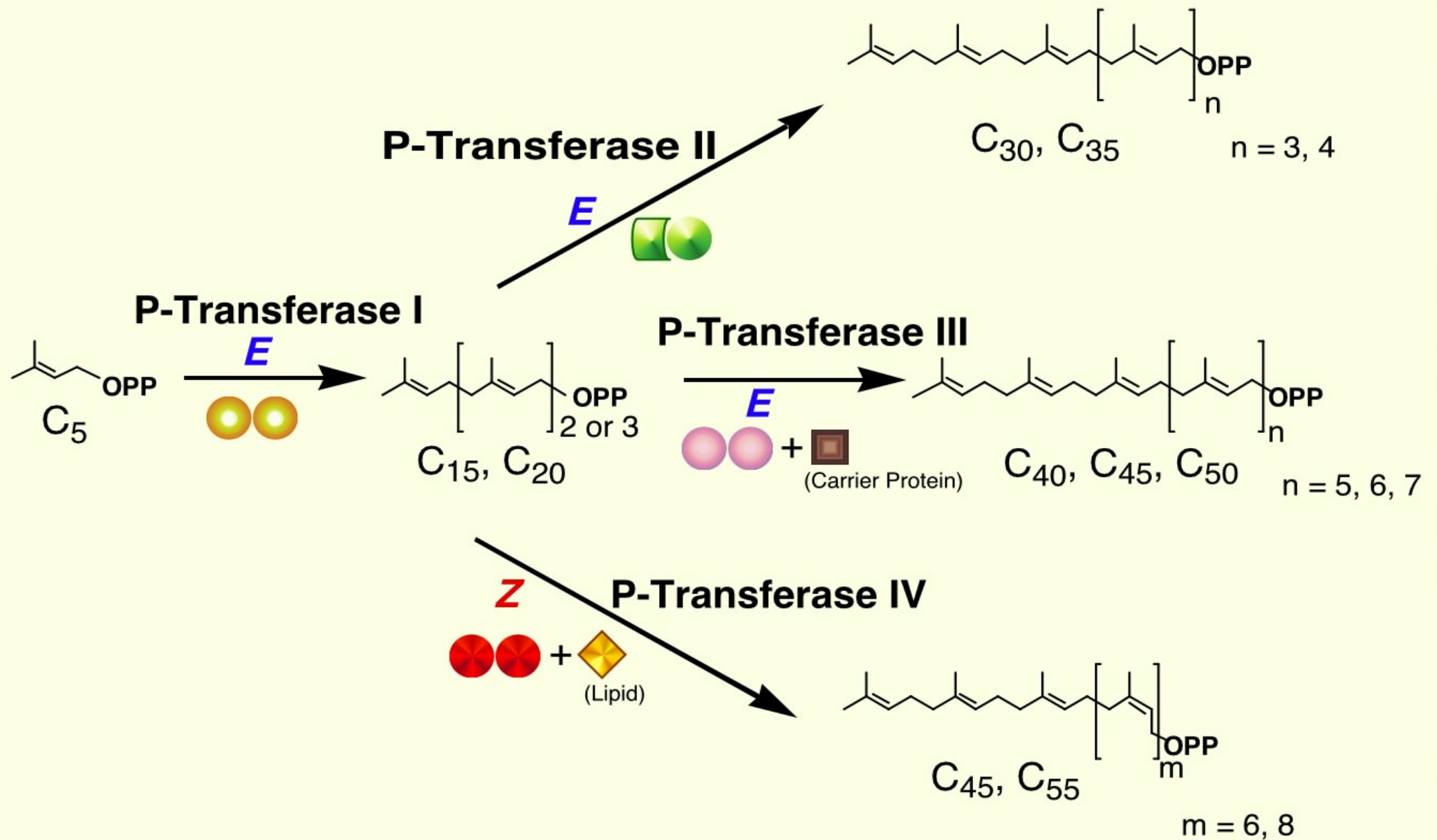




# Catalytic Ranges of Prenyl Chain Elongation by Prenyltransferases so far Characterized

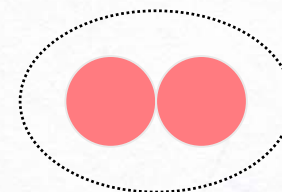
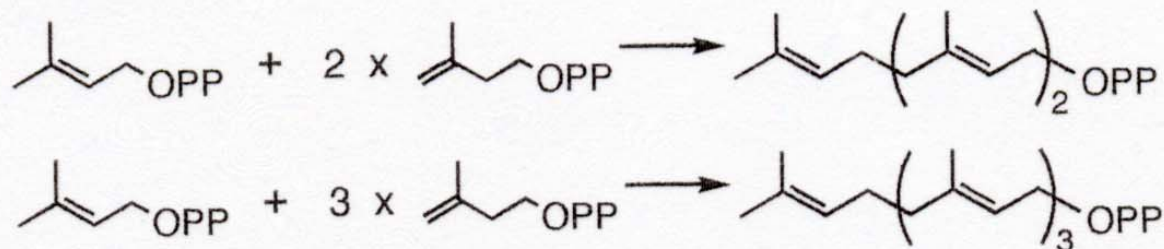


# Classification of Bacterial Prenyltransferases



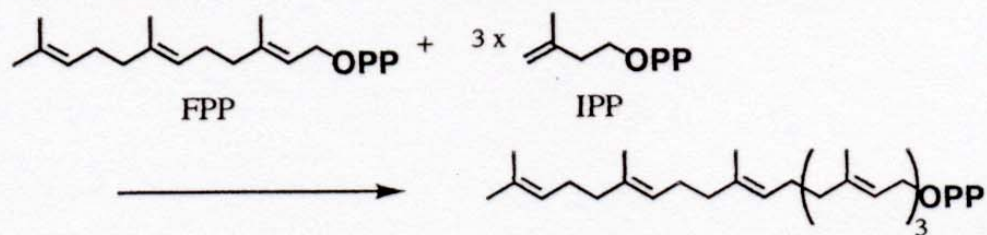


## TRANSFERASE I

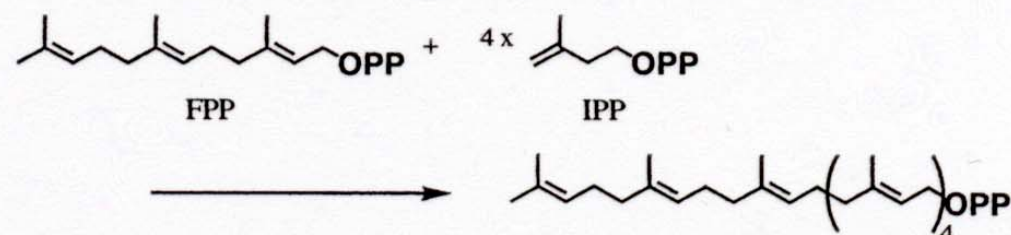


## TRANSFERASE II

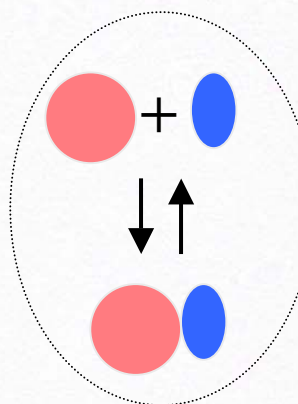
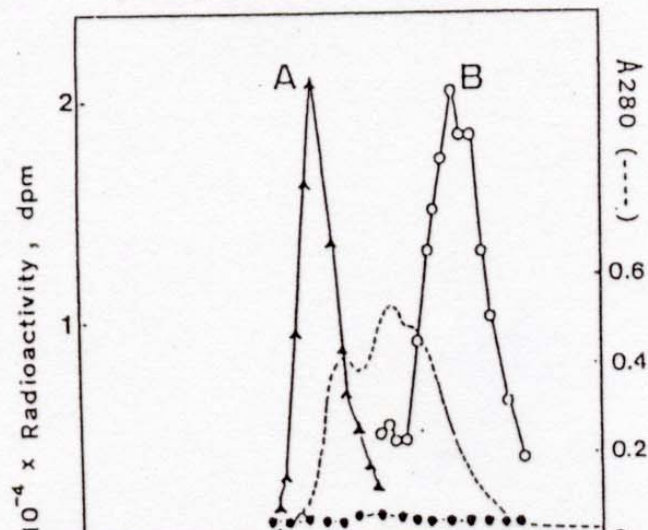
Heptaprenyl Diphosphate Synthase from *Micrococcus luteus* B-P 26



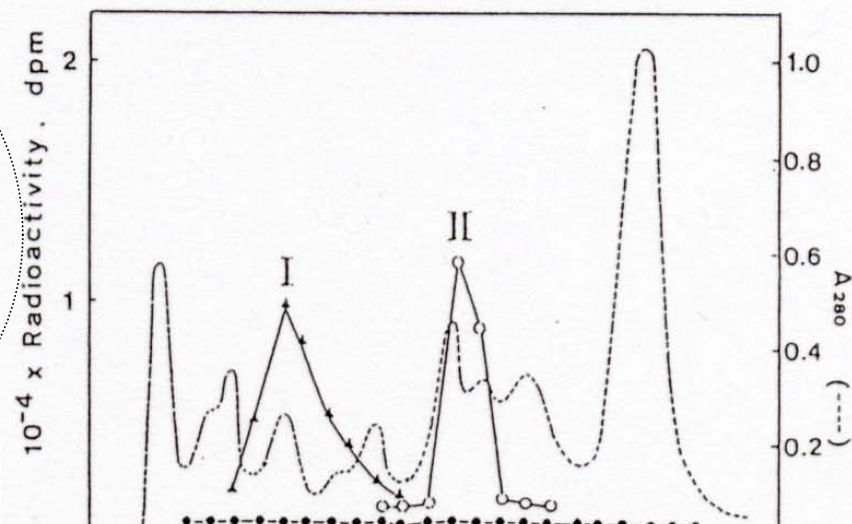
Heptaprenyl Diphosphate Synthase from *Bacillus subtilis*



Hydroxyapatite Chromatography

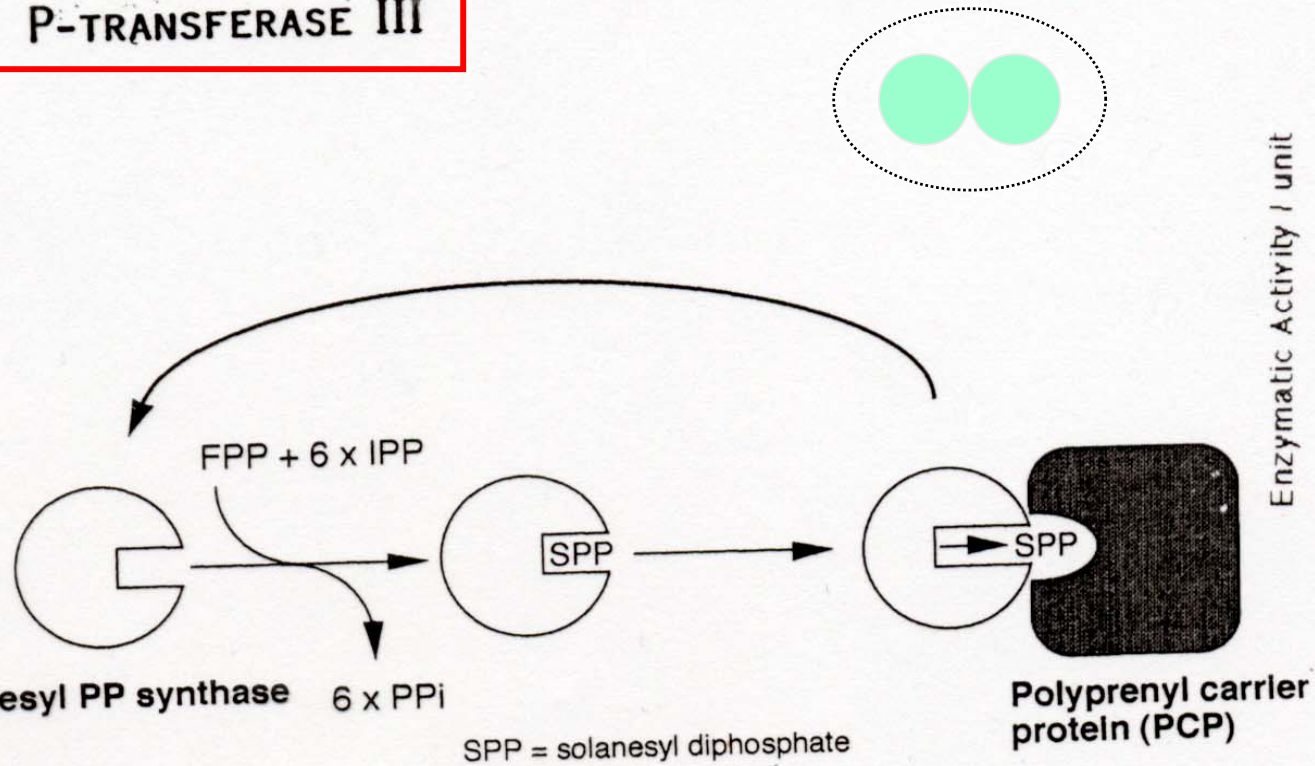


DEAE Sephadex A-50 Chromatography

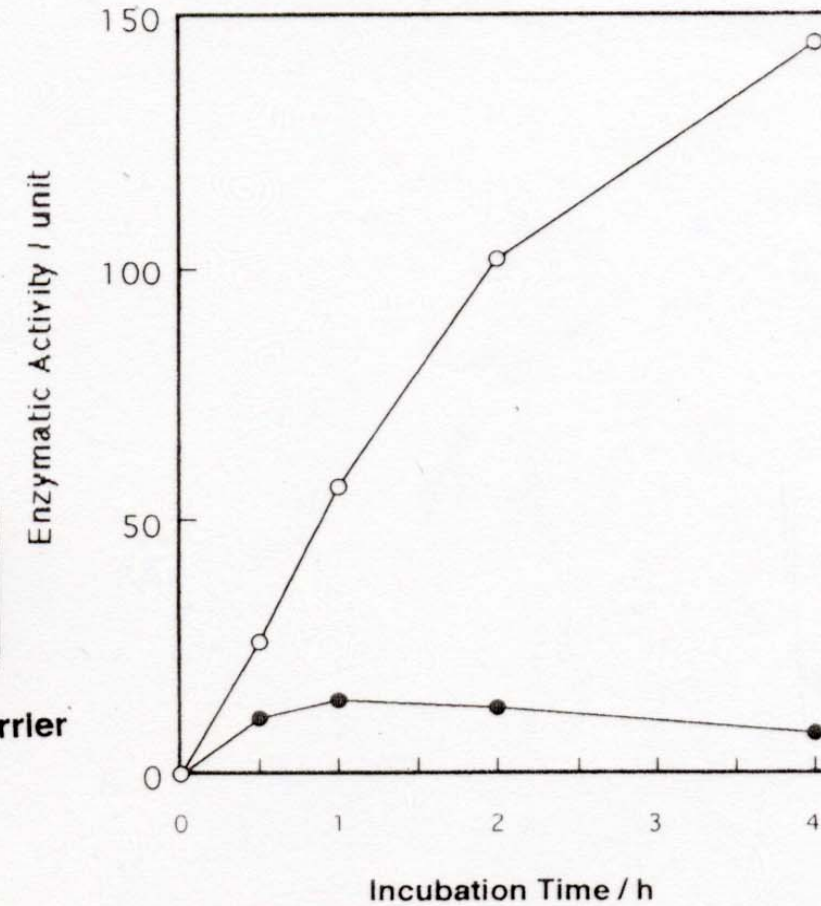




## P-TRANSFERASE III



Turnover of polyprenyl PP synthases dependent on PCP.  
SPP, Solanesyl diphosphate.



Ohnuma, S., et al., J. Biol. Chem., 266, 22706-22713 (1991)

- # Molecular Analysis of Prenyl Chain Elongating Enzymes

1. *trans*-Prenyltransferases
2. *cis*-Prenyltransferases

# Shotgun Cloning of *Bacillus stearothermophilus* Farnesyl Diphosphate Synthase

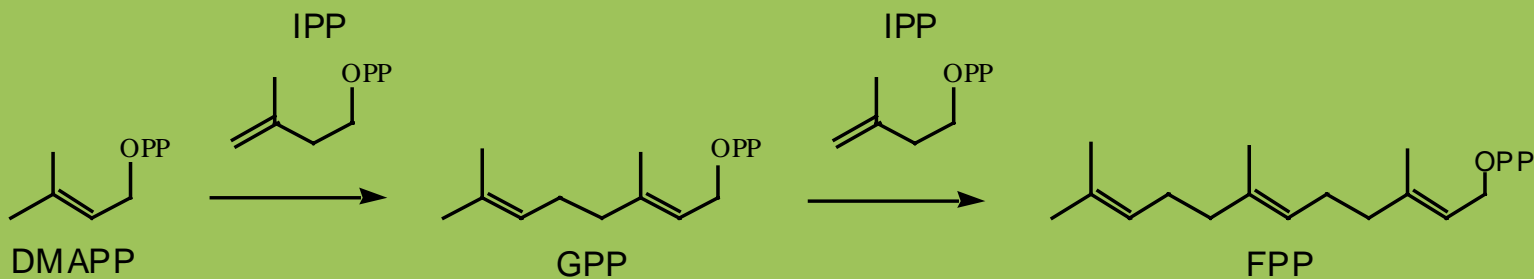
*B. stearothermophilus* genomic library

Cell-free homogenate of each transformant

Heat treatment at 55 °C for 30 min

Screening for heat-stable FPP synthase activity

*fps* gene

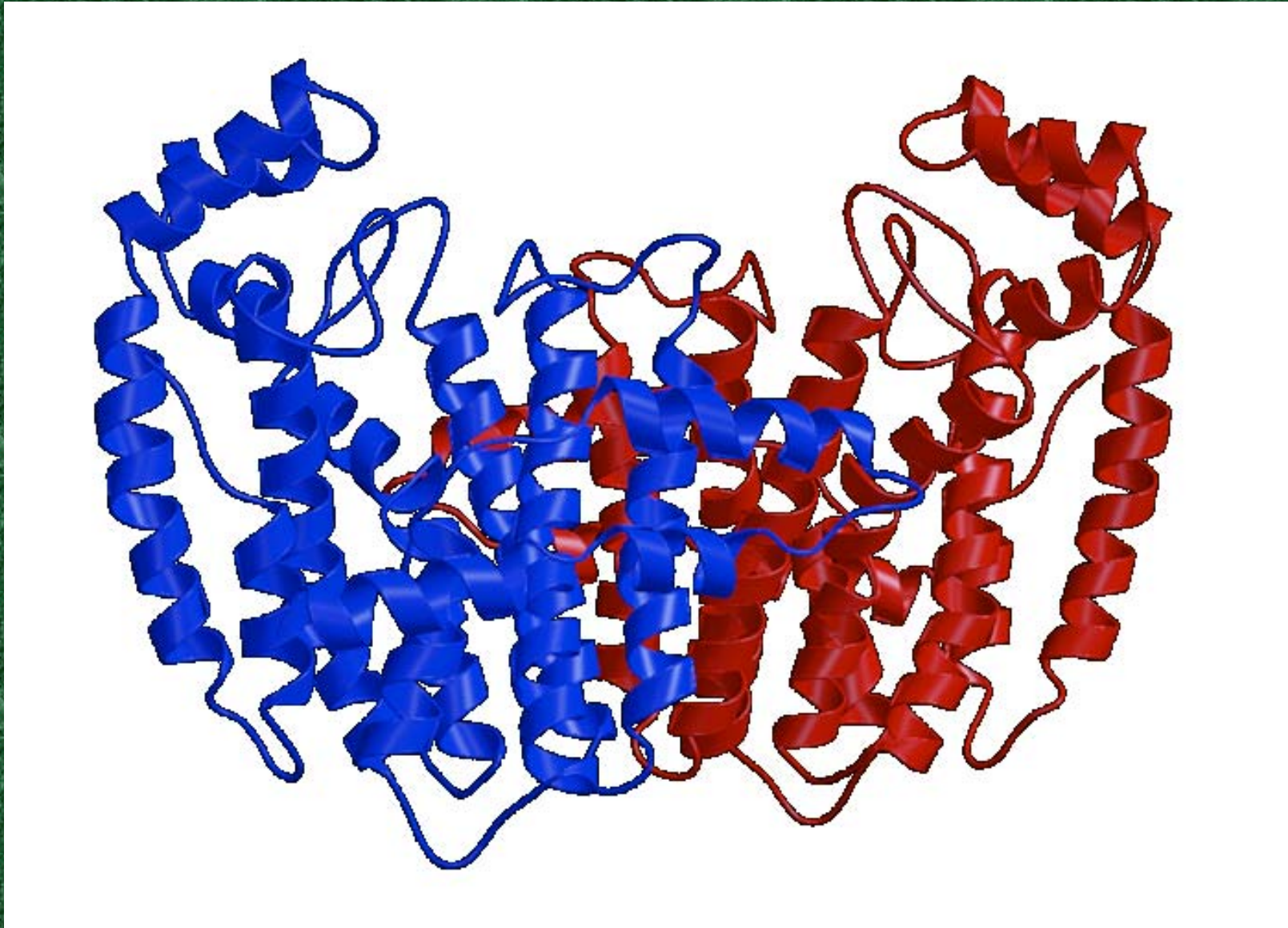


Overproduction, Purification, Crystallization

T. Koyama, S. Obata, M. Osabe, A. Takeshita, K. Yokoyama, T. Nishino, K. Ogura, *J. Biochem.*, **113**, 355 (1993)



# Avian Liver FPP Synthase



Tarshis, L. C. *et al.*, *Biochemistry*, 33, 10871-10877 (1994)



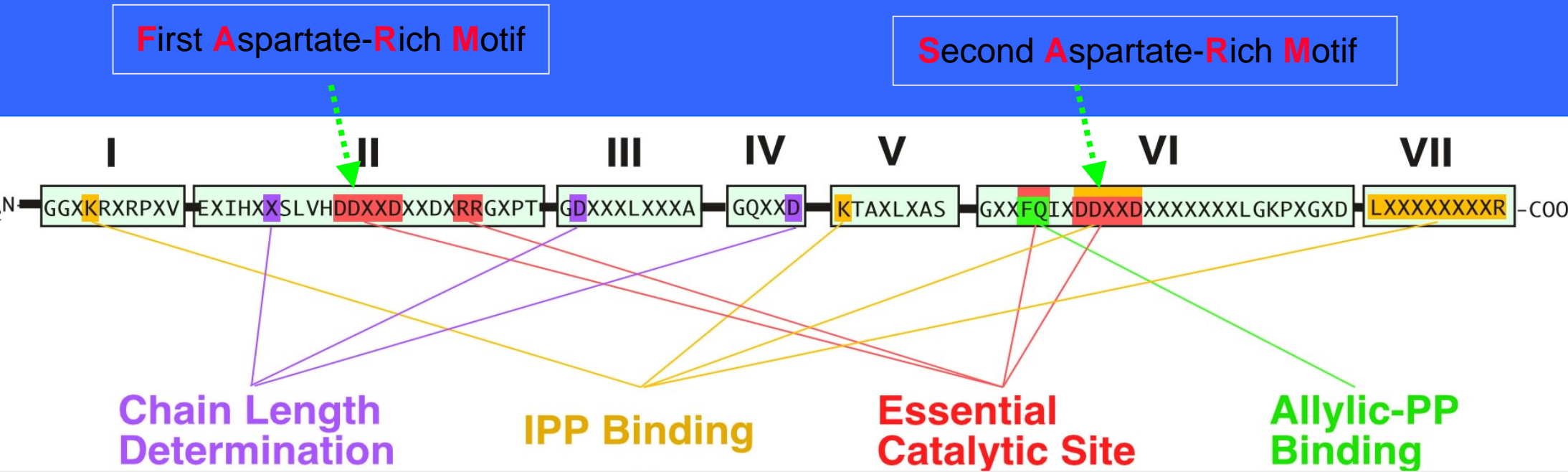
# Amino acid alignments of *trans*-prenyl chain elongating enzymes

N.c. GGPP	:	MAVTSSSPGPAPLSLLSNDDFIAPFNINTKFPSAIVPRTSSNQIPISVAIPSNRISSAGLAATQQAQTRKRKASVAQISLPSMLPTSFSPTYT												
S.c. HexPP	:	MFQRSGAAHHIKLISSRRCRFKSSFAVALNAASKLVTPKILWNNPISLVSKEMNTLAKNIVALIGSGHPVLNKVTSYFFETEGKKVRPLLVLLSRALSEIPMTERNH												
B.s. FPP	:	MA	QLSVEQFLNEQ	K	QAVETALSRYIER	LEGPAKLKKAM	AYSLEA	I	GGKRIRPLL	LLSTVRALGKDPAVGLPVA	II	CAIEMIHTYSLIHDDLPSMDNDDLRRG	KPTN	
E.c. FPP	:	MDFPQQLEACV	KQAN	QAL	SRFIAP	LPFQNTPVVETM	QYGALL		GGKRLRPFL	VYATGHMFGVSTNTLDAPAAAVE		C I HAYSLIHDDLPAAMDDDLRRG	LPTC	
S.c. FPP	:	MASE	KEIRRRERFLNVFPKLV	EELNA	SL	LAYGMPKEACDWAYHS	LNYNTP		GGKLNRG L	SVVDTYAILSNKTV	EQLGQEEYEKVAAILGW	C IELLQAYFLVADD	MDKSITRRG	QPCWYKVEVG
rat FPP	:	MNGDQKLDVHNQE	KQNF	I	QHF	SQIVKV	LTEDELGHPEKGD	AI	TRIKEVLEYNTV	TVVQTFQELVEPRKQDAESLQRALTVGW		C VELLQAFFLVLD	I MDSSHTRRG	QTC
hum. FPP	:	MNGDQNSDVYAQE	KQDFV	QHF	SQIVRV	LTEDEMGHPEIGDAIARLKEVLEYNAI			GGKYNRG L	TVVAFRELVEPRKQDADSLQRAWTVGW		C VELLQAFFLVADD	I MDSSLTRRG	QTC
N.c. GGPP		MAP	QPPQPPPNPDRFATEDFF	SP	SRRTWSEEKEKVL	TGP	YDYLNGHP		GKDIRSQM	VKAFDAWLDPSESLEVIT		KVISM LHTASLLVDDVE	DNSVLRRG	FPVAHSI
S.c. HexPP		LKIDKSDVPEDPIYSKPSQNQLFQRPASSISP	LHILHG	IKPLN	PLTKGPEPLPEETF				D KQ RGIL	PKQRRLA		EIVEMIHTASLLHDDVI	DHSDTRRG	RPS
B.s. FPP		H	KVF	III	GEAMAILAGDGLL	TYAFQLITEIDDERIPPSVRLRLIERLAKAAGPEGMVA	IV	GQAADM	EGEGKTLTLSELEYIHRH	V	KT	GKMLQYSVHAGALIG	GADARQTRELDEFAAHL	
E.c. FPP		HVKF	GEANAILAGDALQ		TLAFSILSDAMPEVSDRDRISMISELASASGIAGMCG		GQALDL	DAEGKHVPLDALERIHRH		KT	GALIRAAVRLGALSAGDKGRRALPVL	DKYAESI		
S.c. FPP		EIAIN	DAFMLEAAIYKLL		KSHFRNEKYYIDITELFHEVTFQTEL		GQLMDL	ITAPEDKVDLSKFSKKHSFIVTF		KT	AYYSFYLPVALAMYVAGITDEKDLKQARDVLIPL			
rat FPP		WYQKP	GIGLDAINDALLL		EAAIYRLKIFYCREQPYLNLLEFLQSSYQTEI		GQTLDL	ITAPQGQVDLGRYTEKRYKSIVKY		KT	AFYSFYLPAAAMYMAGIDGEKEHANALKILLEM			
hum. FPP		WYQKP	GVGLDAINDANLL		EACIYRLKLKYCREQPYLNLIELFLQSSYQTEI		GQTLDL	LTAPQGNVDLVRFTKRYKSIVKY		KT	AFYSFYLPAAAMYMAGIDGEKEHANAKILLEM			
N.c. GGPP		FGIPQ	TINTSNYYVYFAL		QELQK	LKNPKAVSIFSEELLNLHR		GQGMDL	FWRDTLTCPTEDDYLEMVSN		KT	GGLFRLGIKLMQAESRSPVDCVPLVNII		
S.c. HexPP		GNAAF	TNKMVLAGDFLL		GRATVISIRLHNPEVVELMNSIANLVEGEFMQLK		NTSIDA	DIDTIENGHKLLPVPSKKLEVKEHDFRVP	SRQGLQLSHDQIETAFEYIHH		KT	YLKTAALISKSCRAAILSGASPAVIDECYDFGRNL		
B.s. FPP		VI				GLAFQIRDDILDIEGAEKIGKPVGSD	QSNKATYPALLSLAGAKEKLAFHIEAAQRHLRNADVD	VII				GAALAYICELVAARDH*		
E.c. FPP						GLAFQVQDDILDVVGDTATLKGKRGAD	QQLGK STYPALLGLEQARKKARDLIDARQSLKQLAEQSLD					TSALEALADYIITQRNK*		
S.c. FPP						GEYFQIQDDYLDLDFGTPQIGK IGTD	IQDNKCSWVINKALELASAEQRKTLDENYGGKDSVAEAKCKKIFNDLKIEQLYHEEYSIAKDLKAKISQVDES					RGFK		
rat FPP						GEFFQIQDDYLDLDFGDPSTVGK VGTD	IQDNKCSWL	VVQCLLRATPQ	QRQILEENYQKDPKVARVKALYEELDLRSVFFKYEEDSYNRLKSLIEQCSAPLP					
hum. FPP						GEFFQIQDDYLDLDFGDPSTVGK IGTD	IQDNKCSWL	VVQCLQRATPEQYQILKENYGGQEAKEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLP						
N.c. GGPP						GLIFQIADDYHNLWNREYTANKGMCED	LTEGKFSFPVIHSIRSNPSNMQLLNILKQKTGDEEVKRYAVAYMESTGSFEYTRKVIKVLVDRARQMTEDID					DGRGKSGGIHKILDRI		
S.c. HexPP						GICFQLVDDMLDFTVSGKDLGKPSGAD	LKLG	IATAPVLFAWKEDPSLGPLISRNFSERGDVEKIDS					VRHLHNGIAKTKILAEERYDKALQNL	RDSLPESDA

FPP synthases: B. s., *Bacillus stearothermophilus*; E. c., *Escherichia coli*; S. c., *Saccharomyces cerevisiae*; hum, human

N.c. GGPP, GGPP synthase from *Neurospora crassa*; S. c. HexPP, hexaprenyl-PP synthase from *Saccharomyces cerevisiae*

# Roles of the Conserved Regions of *trans*-Prenyl Chain Elongating Enzymes



Koyama, T. et al., Biochemistry, 35, 9533-9538 (1996); Koyama, T. et al., Biochem. Biophys. Res. Commun., 212, 681-686 (1995).; Koyama, T. et al., Can. J. Chem., 72, 75-79 (1994); Koyama, T. et al., Biochemistry, 33, 12644-12648 (1994); Ohnuma, S. et al., J. Biol. Chem., 271, 18831-18837 (1997); Ohnuma, S. et al., J. Biol. Chem., 272, 5192-5198 (1997)



# PRENYLTRANSFERASE GENES CLONED BY US

I	Farnesyl-PP Synthase	(Micrococcus luteus B-P 26) (Bacillus stearothermophilus)	$\text{C}_5 \xrightarrow[2 \times \text{IPP}]{(E)\text{-condensation}} \text{C}_{15}$	<b>HOMODIMER</b> Overproduction, Purification, and Crystallization	T. Koyama, <i>et al.</i> , <i>J. Biochem.</i> <b>113</b> , 355-363 (1993); N. Shimizu <i>et al.</i> , <i>J. Bacteriol.</i> , <b>180</b> , 1578-1581 (1998).
	Geranylgeranyl-PP Synthase	(Hevea brasiliensis)	$\text{C}_5 \xrightarrow[2 \times \text{IPP}]{(E)\text{-condensation}} \text{C}_{20}$	<b>HOMODIMER</b> Overproduction, Purification	A. Takaya <i>et al.</i> , <i>Biochim. Biophys. Acta</i> , <b>1625</b> , 214-220 (2003)
II	Hexaprenyl-PP synthase	(Micrococcus luteus B-P 26)	$\text{C}_{15} \xrightarrow[3 \times \text{IPP}]{(E)\text{-condensation}} \text{C}_{30}$	<b>Dissociable HETERODIMER</b> , Overproduction, Purification Crystallization	N. Shimizu <i>et al.</i> , <i>J. Bacteriol.</i> , <b>180</b> , 1578-1581 (1998).
	Heptaprenyl-PP Synthase	(Bacillus subtilis) (Bacillus stearothermophilus)	$\text{C}_{15} \xrightarrow[4 \times \text{IPP}]{(E)\text{-condensation}} \text{C}_{35}$	<b>Dissociable HETERODIMER</b> , Overproduction, Purification	A. Koike-Takeshita <i>et al.</i> , <i>J. Biol. Chem.</i> , <b>270</b> , 18396-18400 (1995); Y.-W. Zhang <i>et al.</i> , <i>J. Bacteriol.</i> , <b>179</b> , 1417-1419 (1997)
III	Decaprenyl-PP Synthase	(Paracoccus denitrificans)	$\text{C}_{15} \xrightarrow[7 \times \text{IPP}]{(E)\text{-condensation}} \text{C}_{50}$	<b>HOMODIMER</b> , Overproduction, Purification	S. Takahashi <i>et al.</i> , <i>Biochem. Engin. J.</i> , <b>16</b> , 183-190 (2003)

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## **Subunit Interaction of Prenyltransferases**

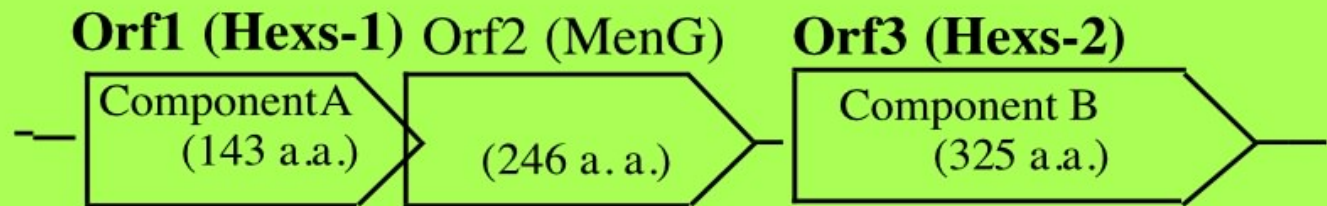
- 1. Dissociable heterodimers for prenyltransferase II**
- 2. Tightly-bound homodimers for prenyltransferase I and -III**



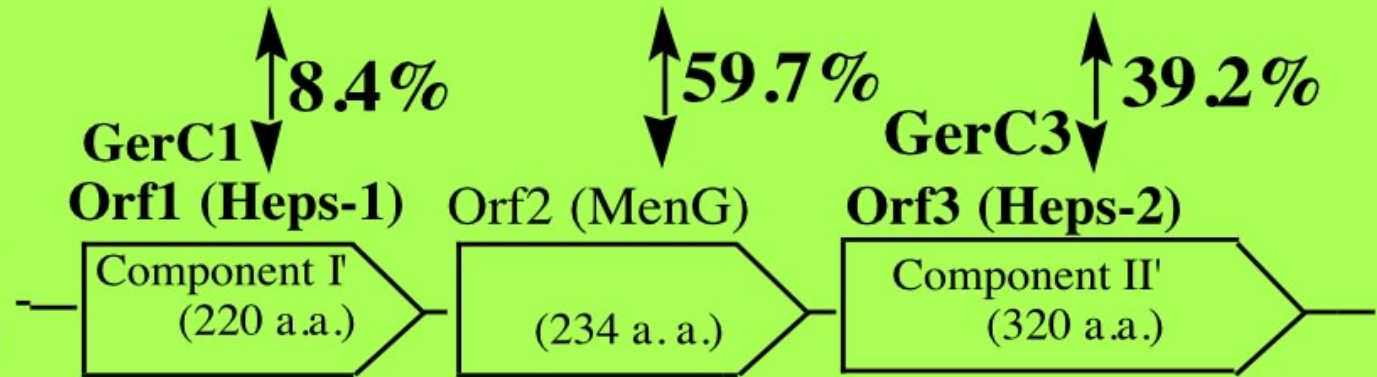
# Prenyltransferase II

## (*E*)-Medium-chain Prenyl Diphosphate Synthase Genes

**HexPP synthase of  
*M. luteus* B-P 26**



**HepPP synthase of  
*B. stearoothermophilus***



**HepPP synthase  
of *B. subtilis***



8.4%

59.7%

39.2%

37.7%

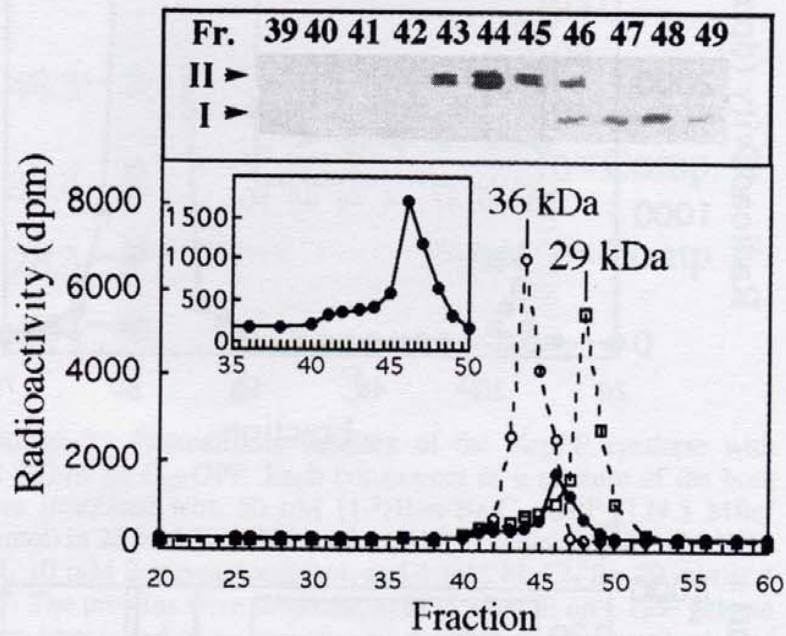
68.7%

65.0%



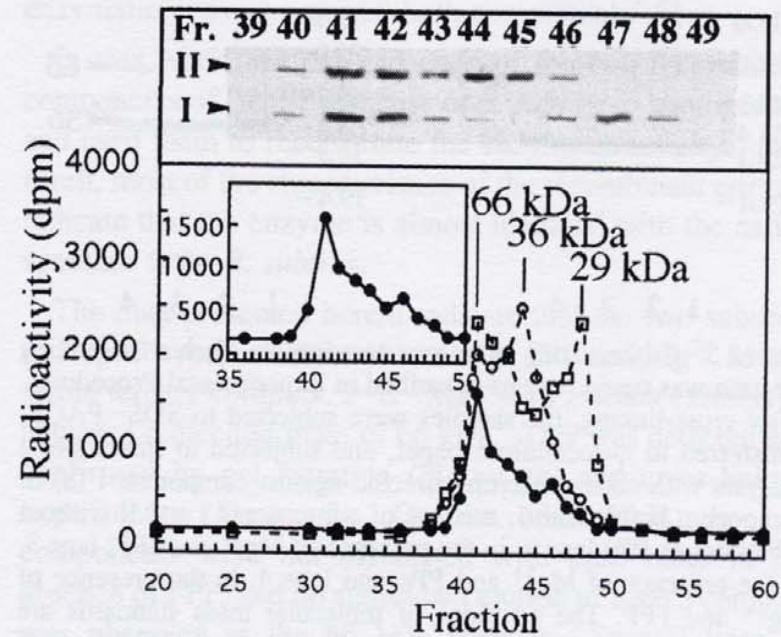
a

No substrate



b

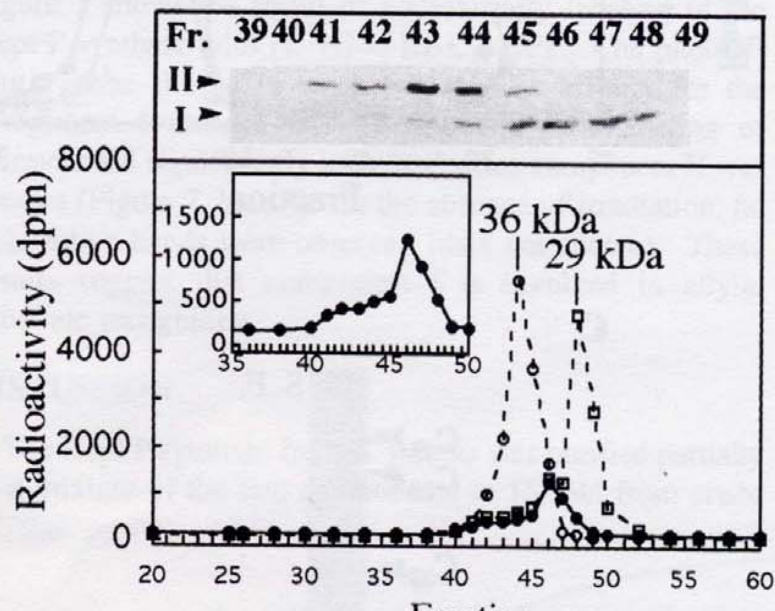
with FPP



## Superdex 200 Gel Filtration Chromatography

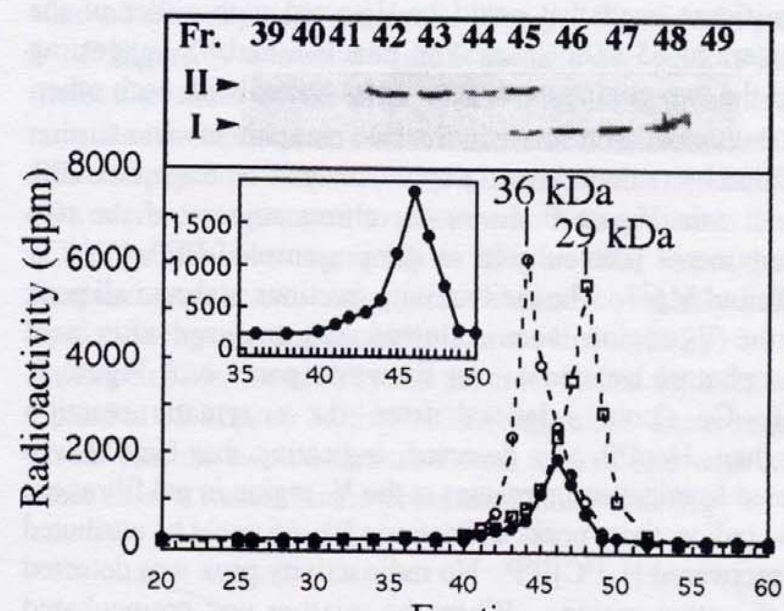
c

with IPP



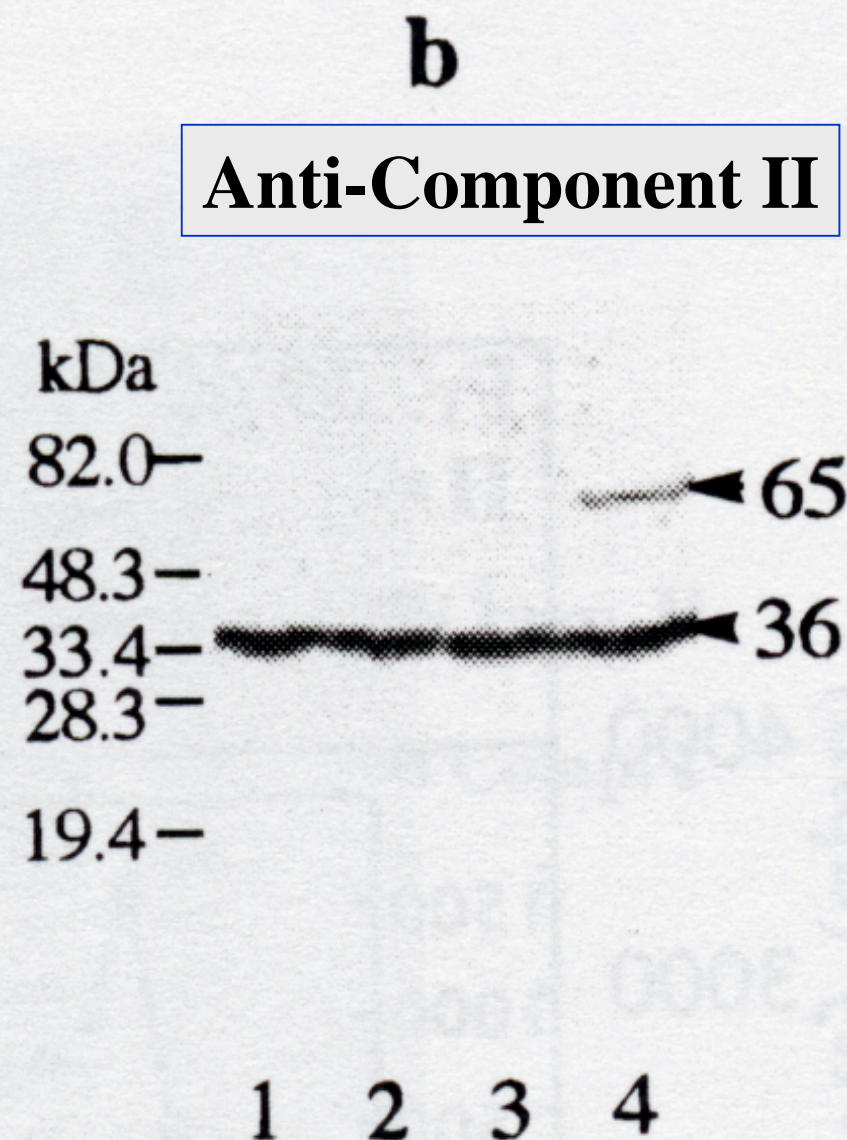
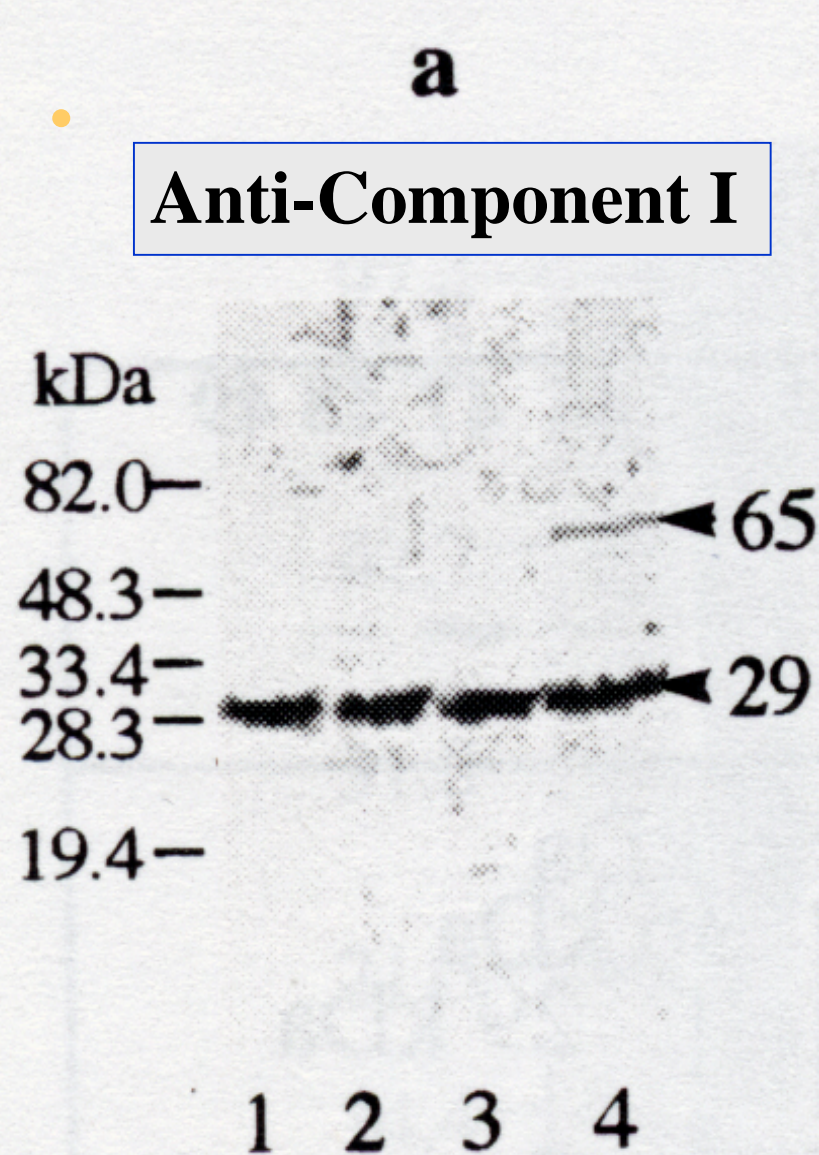
d

with PPi



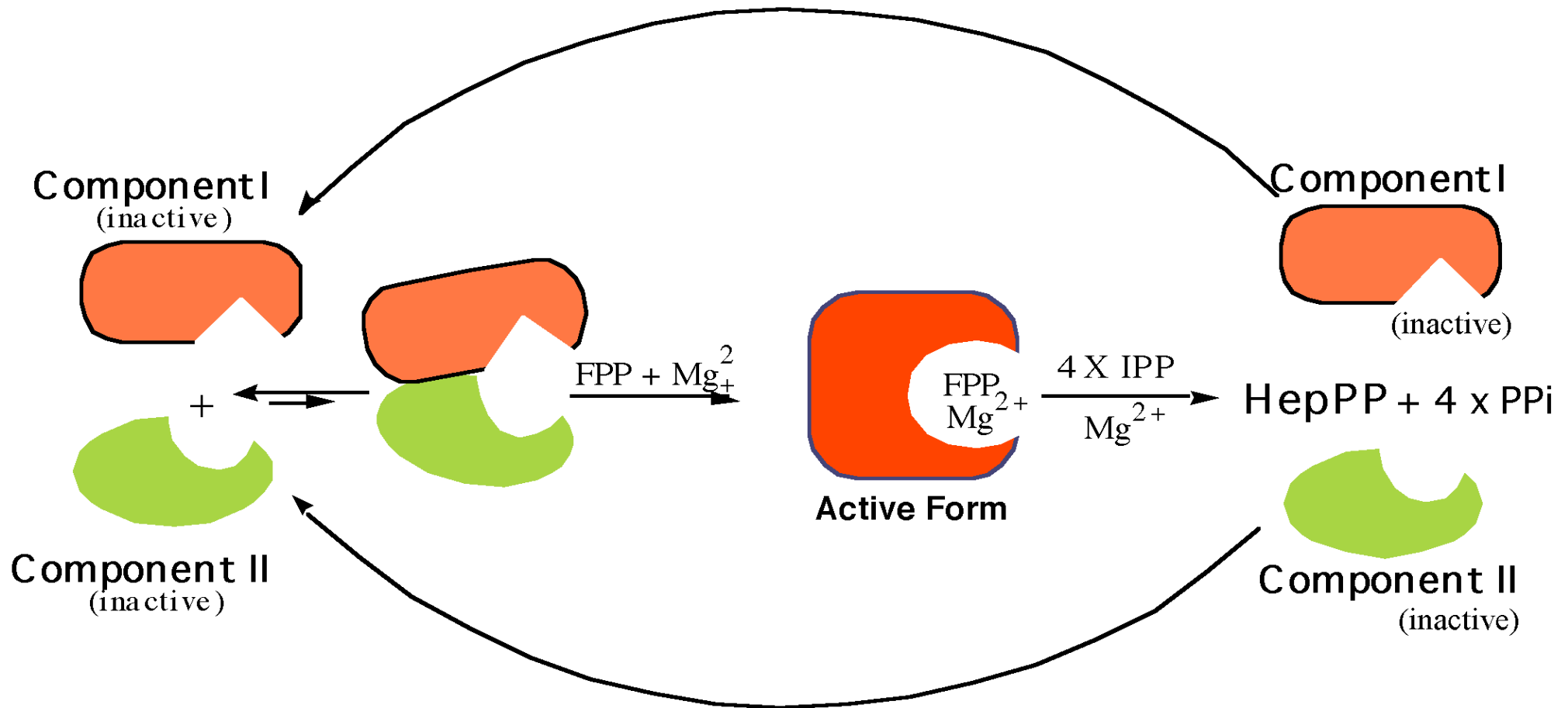


# Cross-linking of the two components with disuccinimidyl suberate



(1, with no substrate; 2, with IPP; 3, with PPI; 4, with FPP)

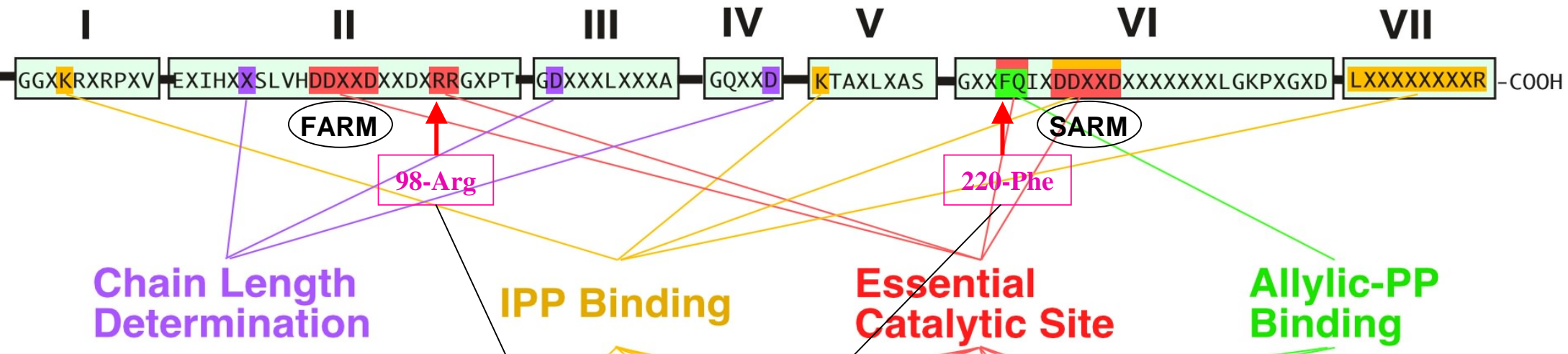
## Hypothetical Mechanism of Hep-PP Synthase Reaction



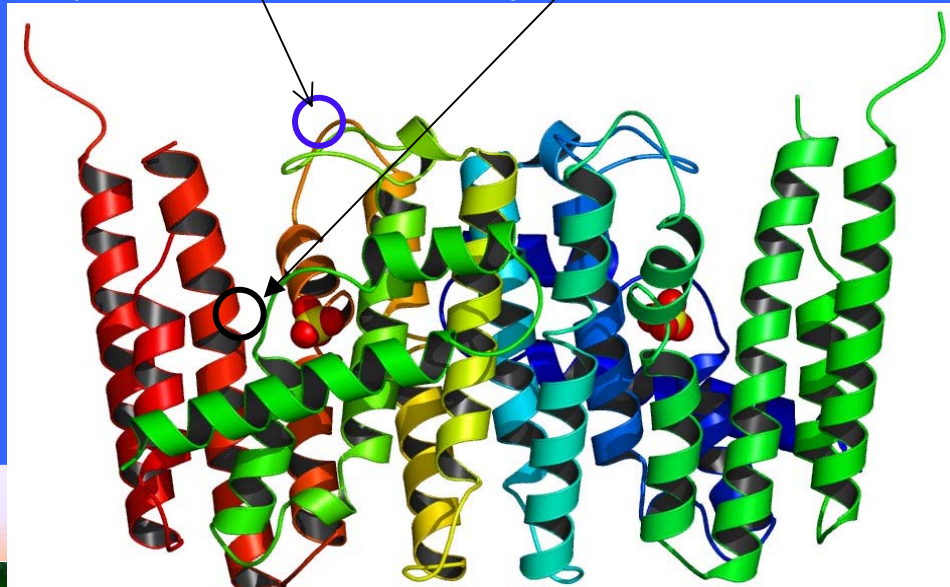


# Amino Acids Critical for the Enzymatic Function

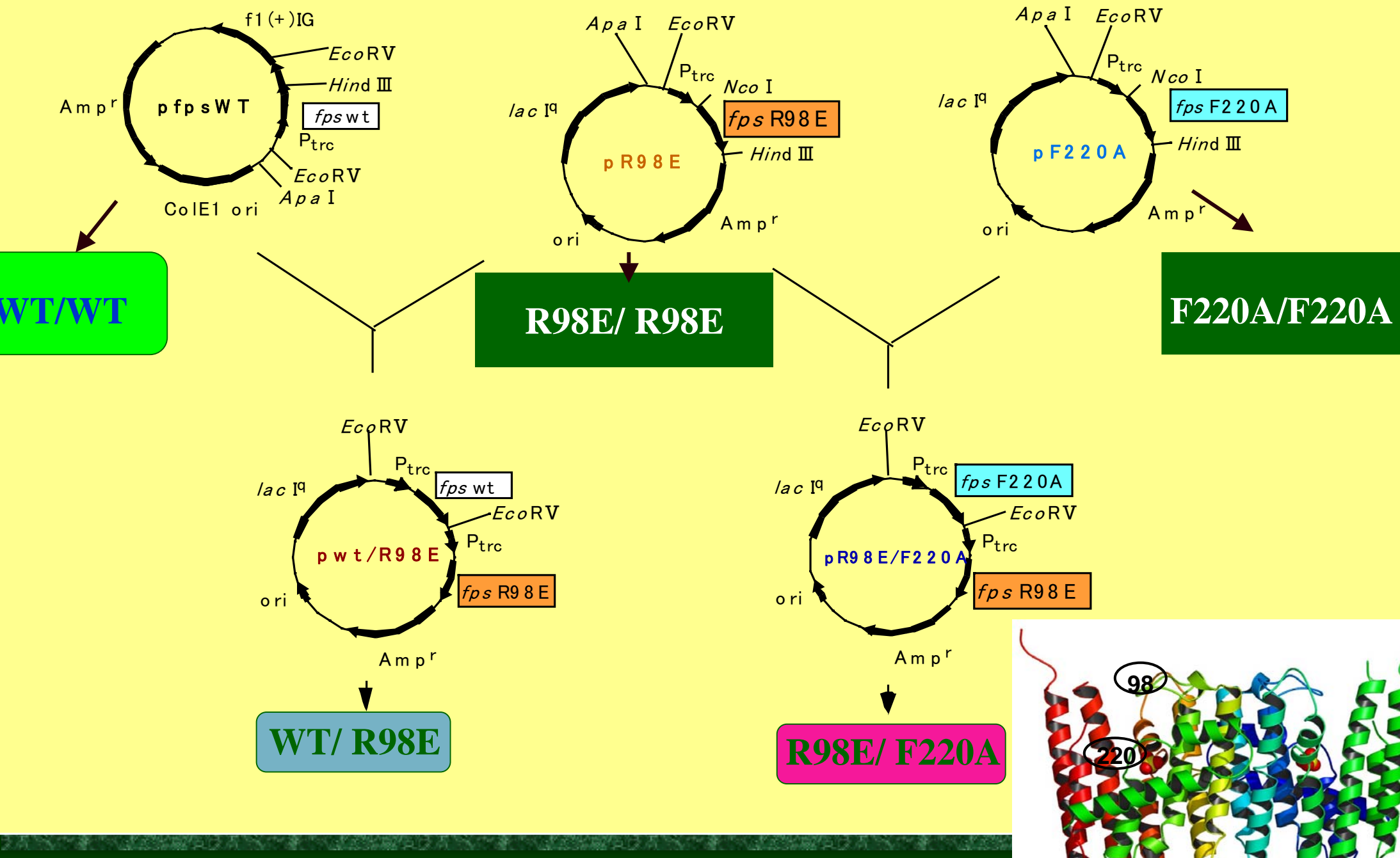
## *E*-Prenyl Diphosphate Synthases



Koyama, T. et al., *Biochemistry*, 35, 9533-9538 (1996); Koyama, T. et al., *Biochem. Biophys. Res. Commun.*, 212, 681-686 (1995).



# Preparation of Hybrid-Type FPP Synthase Heterodimers





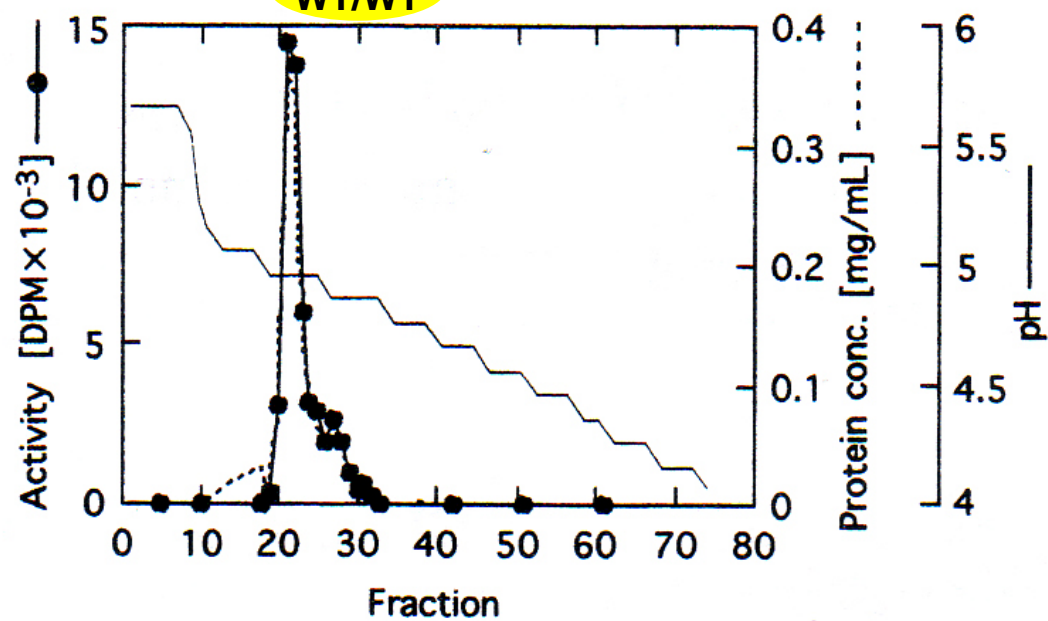
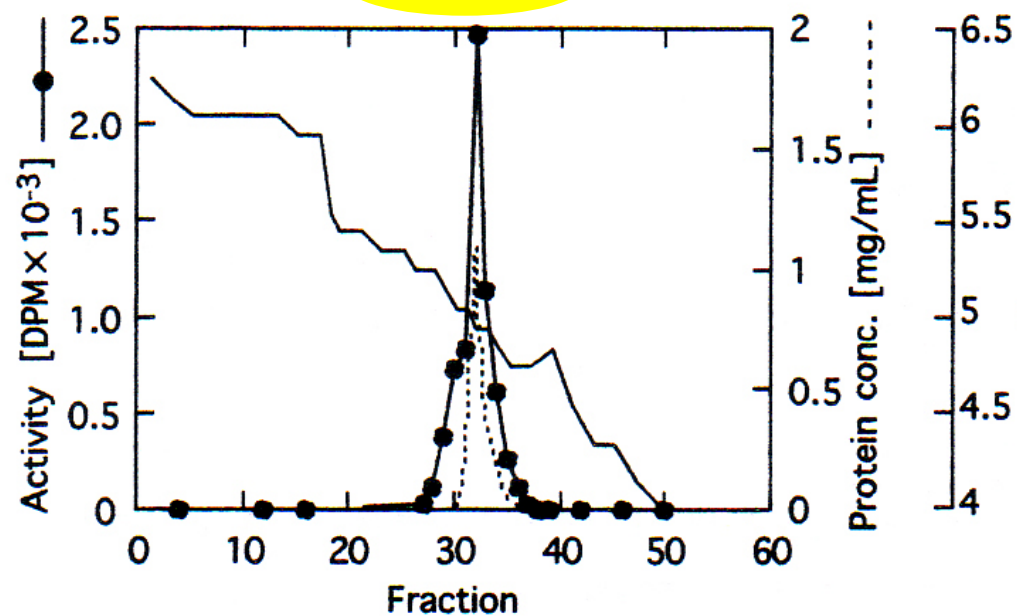
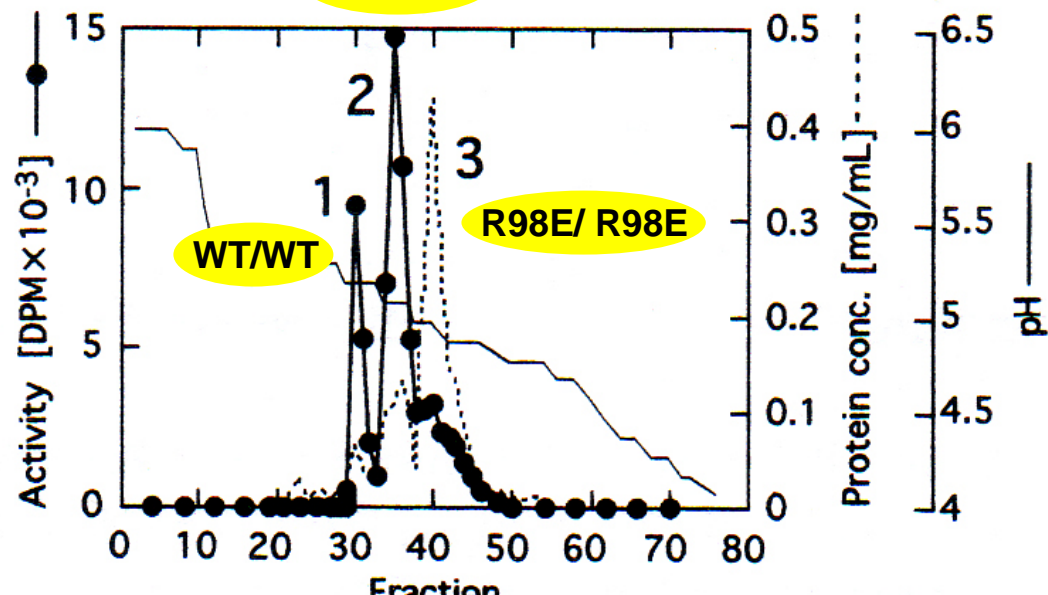
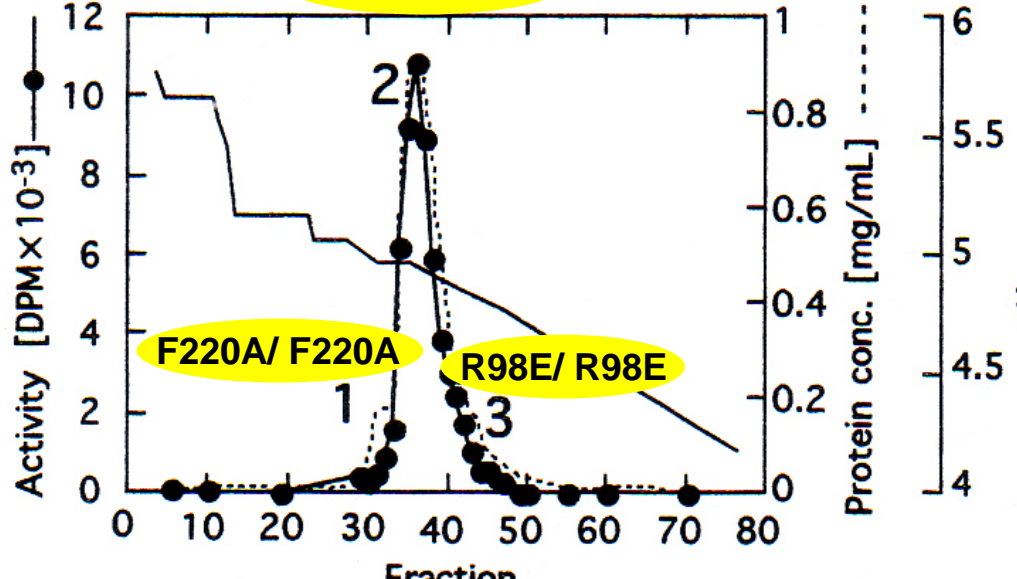
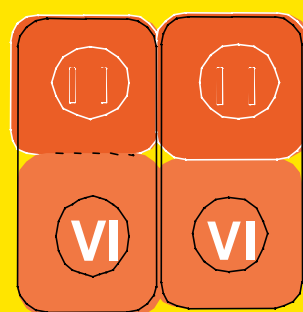
**A****WT/WT****B****R98E/ R98E****C****R98E/WT****D****F220A/ R98E**

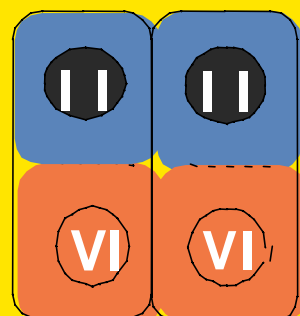
Table 1: Kinetic Parameters of Wild-Type and Mutant Homo- and Heterodimeric FPP Synthases<sup>a</sup>

FPP synthase	$K_m(\text{IPP})^b$ ( $\mu\text{M}$ )	$K_m(\text{GPP})$ ( $\mu\text{M}$ )	$K_m(\text{DMAPP})$ ( $\mu\text{M}$ )	$k_{\text{cat}}^c \times 10^4$ ( $\text{s}^{-1}$ )	$k_{\text{rel}}^d$
wild-type, (WT) <sub>2</sub>	$9.10 \pm 1.23$	$5.54 \pm 1.19$	$11.1 \pm 1.52$	$90700 \pm 3800$	1
(R98E) <sub>2</sub>	$20.1 \pm 2.93$	$12.4 \pm 0.64$	$27.6 \pm 0.20$	$26.3 \pm 0.8$	$2.90 \times 10^{-4}$
(F220A) <sub>2</sub> <sup>e</sup>	$24.9 \pm 3.10$	$3.5 \pm 0.70$	$22.5 \pm 8.6$	$1.18 \pm 0.11$	$1.30 \times 10^{-5}$
WT/R98E	$11.6 \pm 3.21$	$13.5 \pm 0.02$	$19.1 \pm 0.75$	$71100 \pm 4000$	0.784
R98E/F220A	$28.5 \pm 5.37$	$9.84 \pm 0.57$	$17.7 \pm 2.40$	$466 \pm 6.12$	$5.14 \times 10^{-3}$

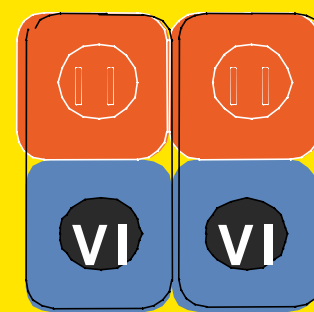
<sup>a</sup> Each value represents the mean  $\pm$  the standard deviation of two determinations. <sup>b</sup> For the reaction with GPP. <sup>c</sup>  $k_{\text{cat}}$  was calculated from the  $V_m$  value for the reaction with IPP and GPP. <sup>d</sup>  $k_{\text{cat}}$  value relative to that of the wild type. <sup>e</sup> Data from ref 22.



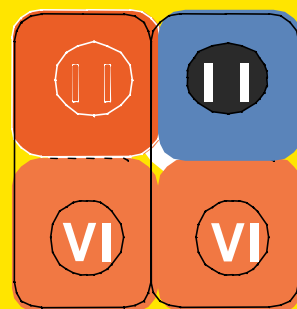
**WT/WT**  
[ $k_{\text{cat}}$ : 9.07]



**R98E/R98E**  
[ $k_{\text{cat}}$ : 0.0026]



**F220A/F220A**  
[ $k_{\text{cat}}$ : 0.00011]



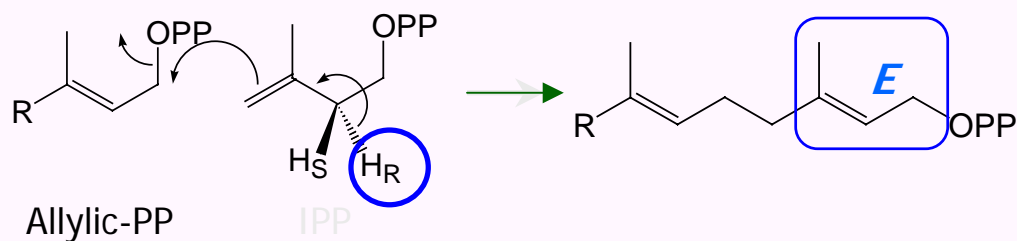
**WT/R98E**  
[ $k_{\text{cat}}$ : 7.11]



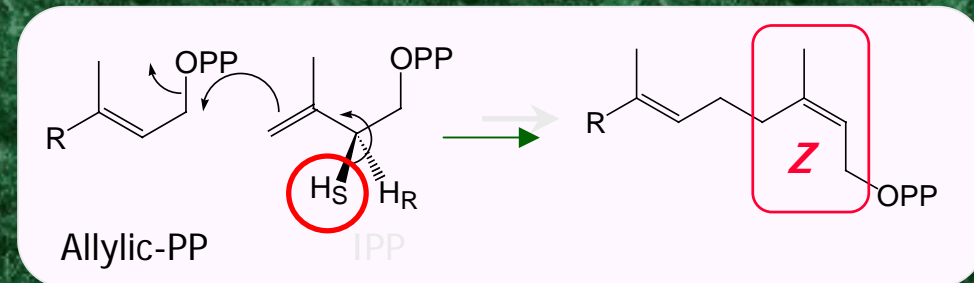
**R98E/F220A**  
[ $k_{\text{cat}}$ : 0.047]



# Cloning of Undecaprenyl Diphosphate Synthase Gene as *cis*-Prenyl Chain Elongating Enzymes



*E*(*trans*)-Prenyltransferase



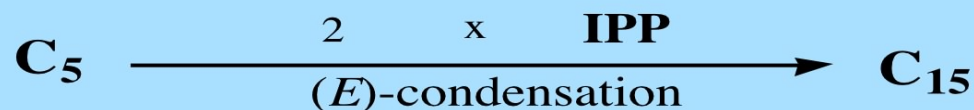
*Z*(*cis*)-Prenyltransferase

# PRENYLTRANSFERASE GENES CLONED BY US

## Farnesyl-PP Synthase

(*Micrococcus luteus* B-P 26)

(*Bacillus stearothermophilus*)

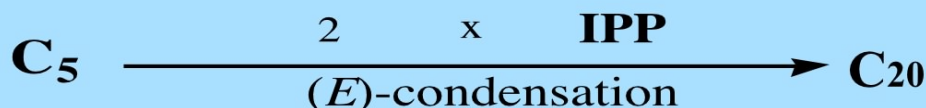


**HOMODIMER** Overproduction,  
Purification, and Crystallization

T. Koyama, *et al.*, *J. Biochem.* **113**, 355-363 (1993); N. Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

## Geranylgeranyl-PP Synthase

(*Hevea brasiliensis*)

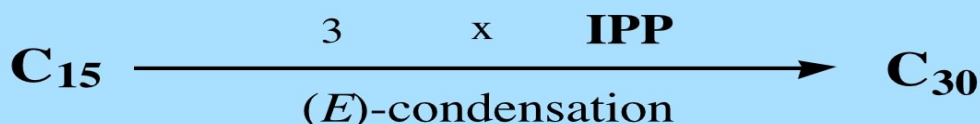


**HOMODIMER** Overproduction,  
Purification

A. Takaya *et al.*, *Biochim. Biophys. Acta*, **1625**, 214-220 (2003)

## Hexaprenyl-PP synthase

(*Micrococcus luteus* B-P 26)



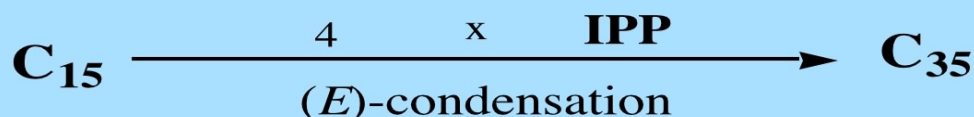
**Dissociable HETERODIMER**,  
Overproduction, Purification  
Crystallization

N. Shimizu *et al.*, *J. Bacteriol.*, **180**, 1578-1581 (1998).

## Heptaprenyl-PP Synthase

(*Bacillus subtilis*)

(*Bacillus stearothermophilus*)

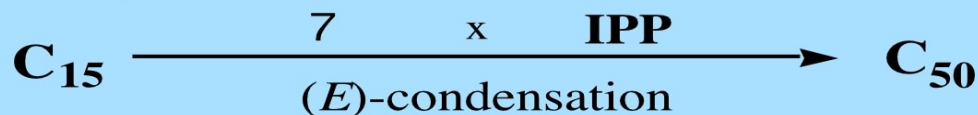


**Dissociable HETERODIMER**,  
Overproduction, Purification

A. Koike-Takeshita *et al.*, *J. Biol. Chem.*, **270**, 18396-18400 (1995); Y.-W. Zhang *et al.*, *J. Bacteriol.*, **179**, 1417-1419 (1997)

## Decaprenyl-PP Synthase

(*Paracoccus denitrificans*)

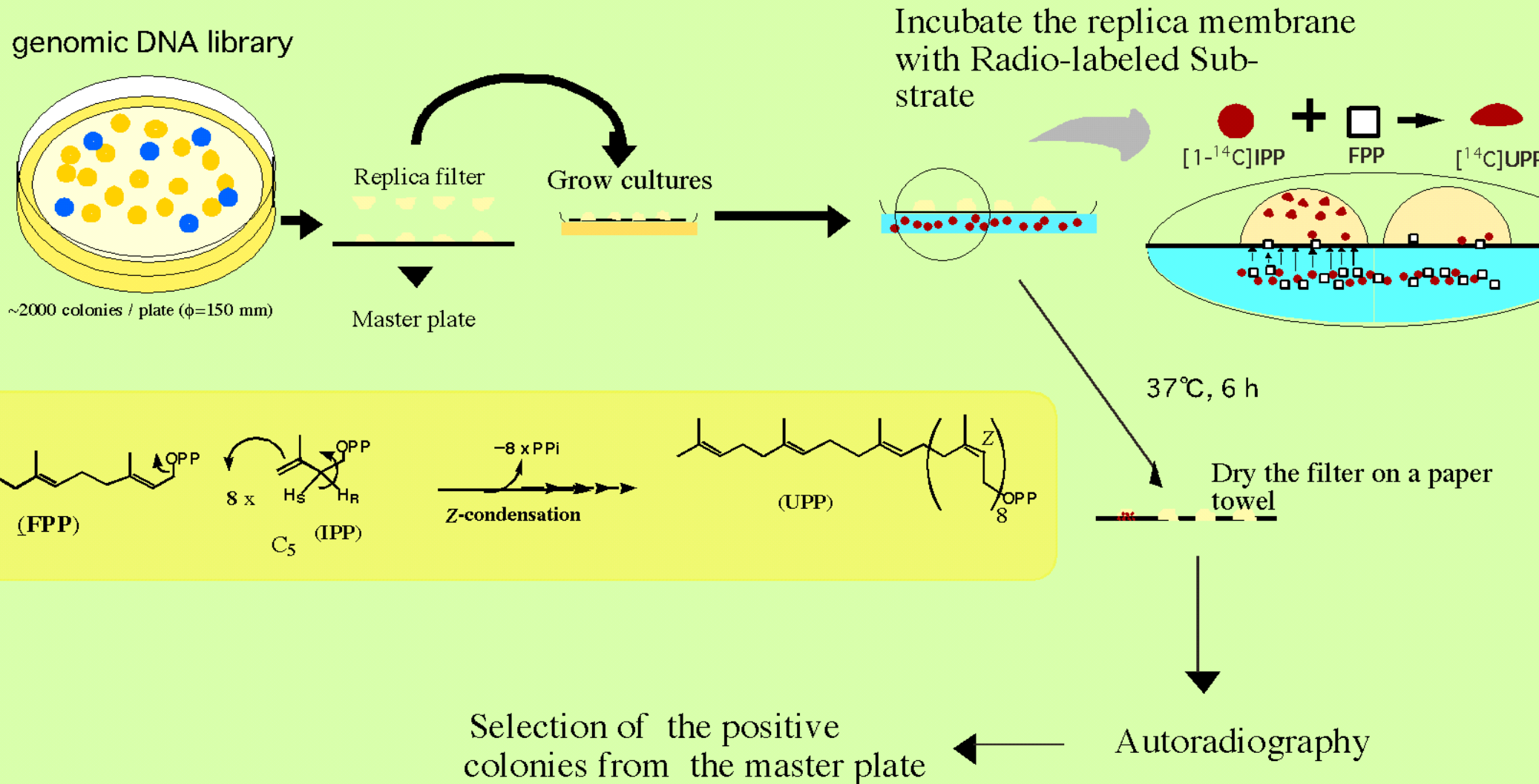


**HOMODIMER**,  
Overproduction, Purification

S. Takahashi *et al.*, *Biochem. Engin. J.*, **16**, 183-190 (2003)



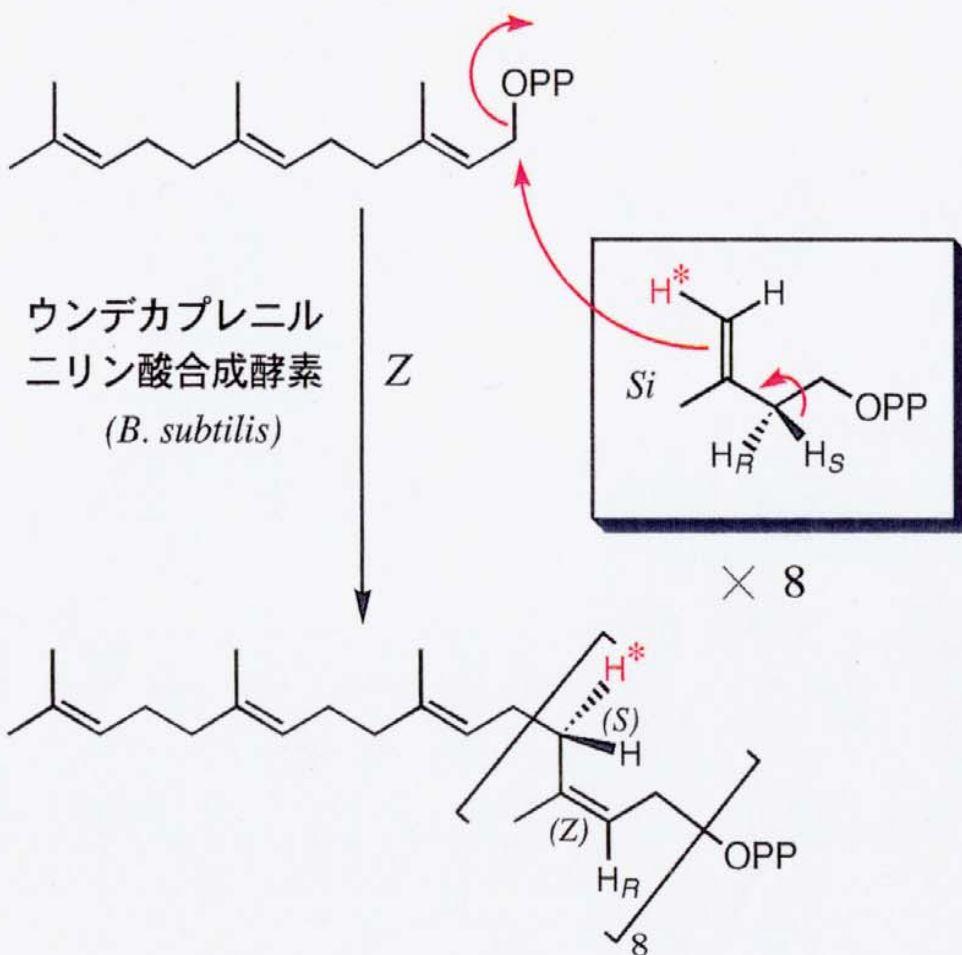
## UPPSynthase Gene Cloning by Colony Autoradiography Method



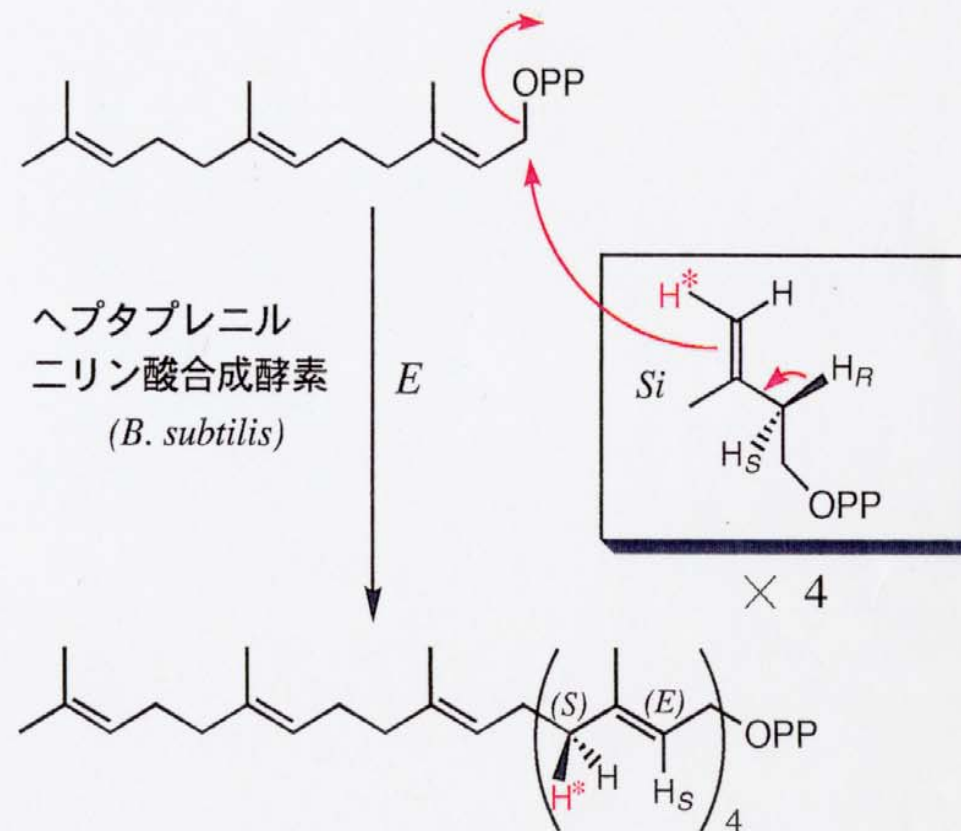
cf. C. R. H. Raetz, *Proc. Nat. Acad. Sci., USA*, **72**, 2272 (1978)

N. Shimizu *et al.*, *J. Biol. Chem.*, **273** (31) 19476-19481 (1998)

## Z 型鎖延長反応



## E 型鎖延長反応



Kobayashi, M. *et al.*, *J. Am. Chem. Soc.*, 107,4588-4589 (1985)

Ito, M. *et al.*, *Biochemistry*, 26, 4745-4740 (1987)



## Amino acid alignments of *cis*-prenyl chain elongating enzymes

[illegible]

Region II

Region III

47 Y E G M Q T V K K I T R Y A S D L G V K Y L T L Y A F S T E N W S R R P K D E V N Y L M K L P G D F L N T F L P - - - E L I E K N V K V E T I G F I D D L P D H T K K A V L E A K E K T K H N T G L T L V F

59 R M G A A K I A E M L R W C H E A G I E L A T V Y L L S T E N L Q R D P D E L A A L E I E I T D V V E E I C A P - - - A N H W S - - V R T V G D L G L I G E E P A R R L R G A V E S T P E V A S F H V N V

94 K M G E A V V I D I A C G A I E L G I K W L S L Y A F S T E N W K R S P E E V R F L M G F N R D V V R R R R D - - - T L K K L G V R I R W V G S R P R L W R S V I N E L A V A E E M T K S N D V I T I N Y

44 K A G A K S V R R A V S F A A N N G I E A L T L Y A F S T E N W N R P A Q E V S A L M E L F V W A L D S E V K - - - S L H R H N V R L R I G D T S R F N S R L Q E R I K S E A L T A G N T G L T L N I A

97 E A G A K R L D I A E L C F E L G V H T V S A F A F S T E N W G R D K I E I D N L M S L I Q H Y R N K S N I K - - - F H R S E V R V S V I G N K T K I P E S L L K E I H E I E A T K G Y K N K H L I M

58 E A G F V S M S R I L E L C Y E A G V D T A T V E A F S I E N F K R S S R E V E S L M T L A R E R I R Q I T E R G E - - L A C K Y G V R I K I I G D L S L L D K S L L E D V R V A V E T T K N N K R A T L N I C

52 S Q G F N K L A E T L R W C L N L G I L E V T V Y A F S I E N F K R S K S E V D G L M D L A R Q K F S R L M E E K E - - K L Q K H G V C I R V L G D L H L L P L D Q E L I A Q A V Q A T K N Y N K C F L N V

59 K A G F L A L L N V L T Y C Y E L G V K Y A T I Y A F S I D N F R R K P H E V Q Y Y V M D L M L E K I E G M I E E S - - I I N A Y D I C V R F V G N L K L L S E P V K A A D K I M R A T A N N S K C V L L I A

59 K A G F L A L L N V L T Y C Y E L G V K Y A T I Y A F S I D N F R R K P H E V Q Y Y V M N L M L E K I E G M I E E S - - I I N A Y D I C V R F V G N L K L L D E P L K T A A D K I M R A T A N N S K C F V L L I A

93 E A G G L T L L T L L Y I C K R L G V K C V S A Y A F S I E N F N R P K E E V D T L M N L F T V K L D E F A K R A K D Y K D P L Y G S K I R I V G D Q S L L S P E M R K K I K K V E E I T Q D G D D F T L F I

	Region IV																														Region V																																																																									
146	L	N	Y	G	G	R	K	E	I	I	S	A	V	Q	L	I	A	E	R	Y	K	S	G	-	-	-	-	-	-	-	-	-	-	E	I	S	L	D	E	T	S	E	T	H	F	N	E	Y	L	F	T	-	-	-	-	-	-	A	N	M	P	D	P	E	L	L	I	R	T	S	G	E	E	R	L	S	N	F	L	I	W	Q	C	S	Y	S	-	-	-	E	F	V	F	I	D									
156	V	G	Y	G	G	R	R	E	I	V	D	A	V	R	A	L	L	S	K	E	L	A	N	-	-	-	-	-	-	-	-	-	-	-	G	A	T	A	E	E	L	V	D	A	V	T	N	E	G	I	S	E	N	L	Y	T	S	G	Q	P	D	P	E	L	L	I	R	T	S	G	E	E	R	L	S	N	F	L	I	W	Q	S	A	Y	S	-	-	-	E	M	V	F	I	D										
193	V	N	Y	G	G	R	T	E	I	T	E	A	T	R	E	I	A	E	R	V	A	A	G	-	-	-	-	-	-	-	-	-	-	-	R	L	N	P	E	R	I	T	E	S	T	I	A	R	H	L	Q	R	-	-	-	-	P	D	I	P	D	V	L	F	L	I	R	T	S	G	E	Q	R	S	N	F	L	I	W	Q	A	A	Y	A	-	-	-	E	Y	I	F	Q	D											
143	A	N	Y	G	G	R	W	D	I	V	Q	G	V	R	Q	L	A	E	K	V	Q	Q	G	-	-	-	-	-	-	-	-	-	-	-	N	L	Q	P	D	Q	I	D	E	E	M	L	N	Q	H	V	C	M	-	-	-	-	H	E	L	A	P	V	D	L	V	I	R	T	S	G	E	H	R	I	S	N	F	L	I	W	Q	I	A	Y	A	-	-	-	E	L	Y	F	T	D										
197	V	D	Y	S	G	K	F	D	I	M	H	A	C	K	S	L	V	K	K	S	E	K	G	-	-	-	-	-	-	-	-	-	-	-	L	I	R	E	D	V	D	E	A	L	I	E	R	E	L	L	T	N	C	-	-	-	S	D	F	P	S	P	D	L	M	I	R	T	S	G	E	Q	R	I	S	N	F	L	I	W	Q	L	A	Y	A	-	-	-	E	L	F	E	S	P										
160	F	P	Y	T	G	R	E	I	L	H	A	M	K	E	T	I	V	Q	H	K	K	G	-	-	-	-	-	-	-	-	-	-	-	-	-	G	A	A	D	E	S	T	L	E	S	H	L	Y	T	A	G	-	-	-	-	V	P	P	L	D	L	L	I	R	T	S	G	V	S	R	L	S	D	E	L	I	W	Q	A	S	K	G	V	R	I	E	L	L	D															
154	F	A	Y	T	S	R	H	E	I	S	N	A	V	R	E	M	A	W	G	V	E	Q	G	-	-	-	-	-	-	-	-	-	-	-	-	L	L	D	P	S	D	I	S	E	S	L	D	K	C	L	Y	T	N	R	-	-	-	-	S	P	H	P	D	I	L	I	R	T	S	G	E	V	R	L	S	D	E	L	L	I	W	Q	T	S	H	S	-	-	-	C	L	V	E	Q	P									
161	V	C	Y	T	S	T	S	D	E	I	V	H	A	V	E	S	S	E	L	N	S	N	E	V	C	N	N	Q	E	L	E	E	A	N	A	T	G	S	G	T	V	I	Q	I	E	N	M	E	S	Y	S	G	I	K	L	V	D	L	E	K	N	T	Y	I	-	-	-	-	N	P	Y	P	D	V	L	I	R	T	S	G	E	T	R	L	S	N	F	L	I	W	Q	T	T	N	C	-	-	-	I	L	Y	S	P	H
161	V	C	Y	T	S	T	S	D	E	I	V	H	A	V	E																																																																																									

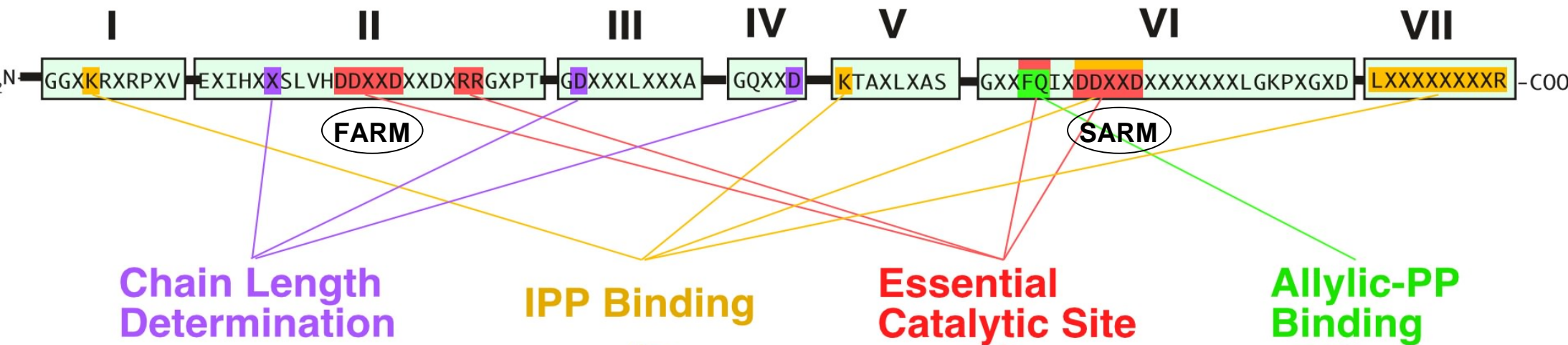
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 237 H W P A E R H V D F L R A L R D Y S A R H R S Y G R - - - - -  
 270 L W P D Y D R R D L W A A C E E Y A S R T R R F G S A - - - - -  
 220 L W P D F D E Q D F E G A L N A F A N R E R R F G G T E P G D E T A - - - - -  
 276 F W P D F D K D K L L E A L A S Y Q R R E R R F G C R V - - - - -  
 235 L W P E E G P I R M A W I L L K F S F H K S F L N K E Y R L E E - - - - -  
 231 L W P E Y T F W N L F E A I L Q F Q M N H S V L Q K A R D M Y A E E R K R Q Q L E R D Q A T V T E Q L L R E G L Q A S G D A Q L R R T R L H K L S A R R E E R V Q G F L Q A L E L K R A D W L A R L G T A S A - - - - -  
 259 L W P E I G L R H V V W A V I N F Q R H Y S Y L E K H K E Y L K - - - - -  
 253 L W P E I G L R H V V W A V I N C Q R H Y S Y L E K H K E Y L K - - - - -  
 274 L W P N E S F F A M Y L M I L K W S F F S T I O K Y N E K N H S L F E K I H E S V P S I F K K K K T A M S L Y N F P N P P I S V S V T G D E - - - - -

M. luteus: *M. luteus* B-P 26 UPP synthase  
Rv1086: *M. tuberculosis* Rv1086 (FPP synthase)  
Rv2361: *M. tuberculosis* Rv2361 (DecPP synthase)  
E. coli: *E. coli* UPP synthase  
A. thaliana: *A. thaliana* DedoIPP synthase  
Rer2p: *S. cerevisiae* Rer2p (DedoIPP synthase)  
HDS: *H. sapiens* HDS (DedoIPP synthase)  
HRT1: *H. brasiliensis* HRT1 (cis-prenyltransferase in latex)  
HRT2: *H. brasiliensis* HRT2 (cis-prenyltransferase in latex)  
Str1p: *S. cerevisiae* Str1p (DecoIPP synthase)

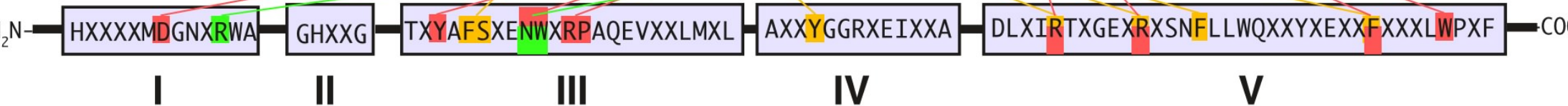


# Comparison of Conserved Regions of *E*- and *Z*-Prenyl Diphosphate Synthases

## *E*-Prenyl Diphosphate Synthases

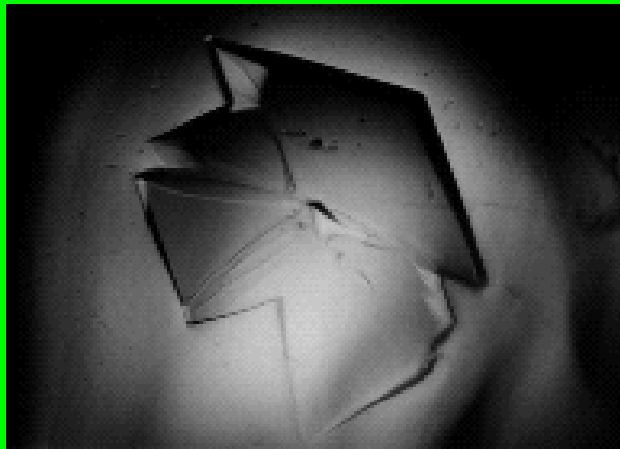


## *Z*-Prenyl Diphosphate Synthases





# Crystal of UPS from *Micrococcus luteus* B-P 26

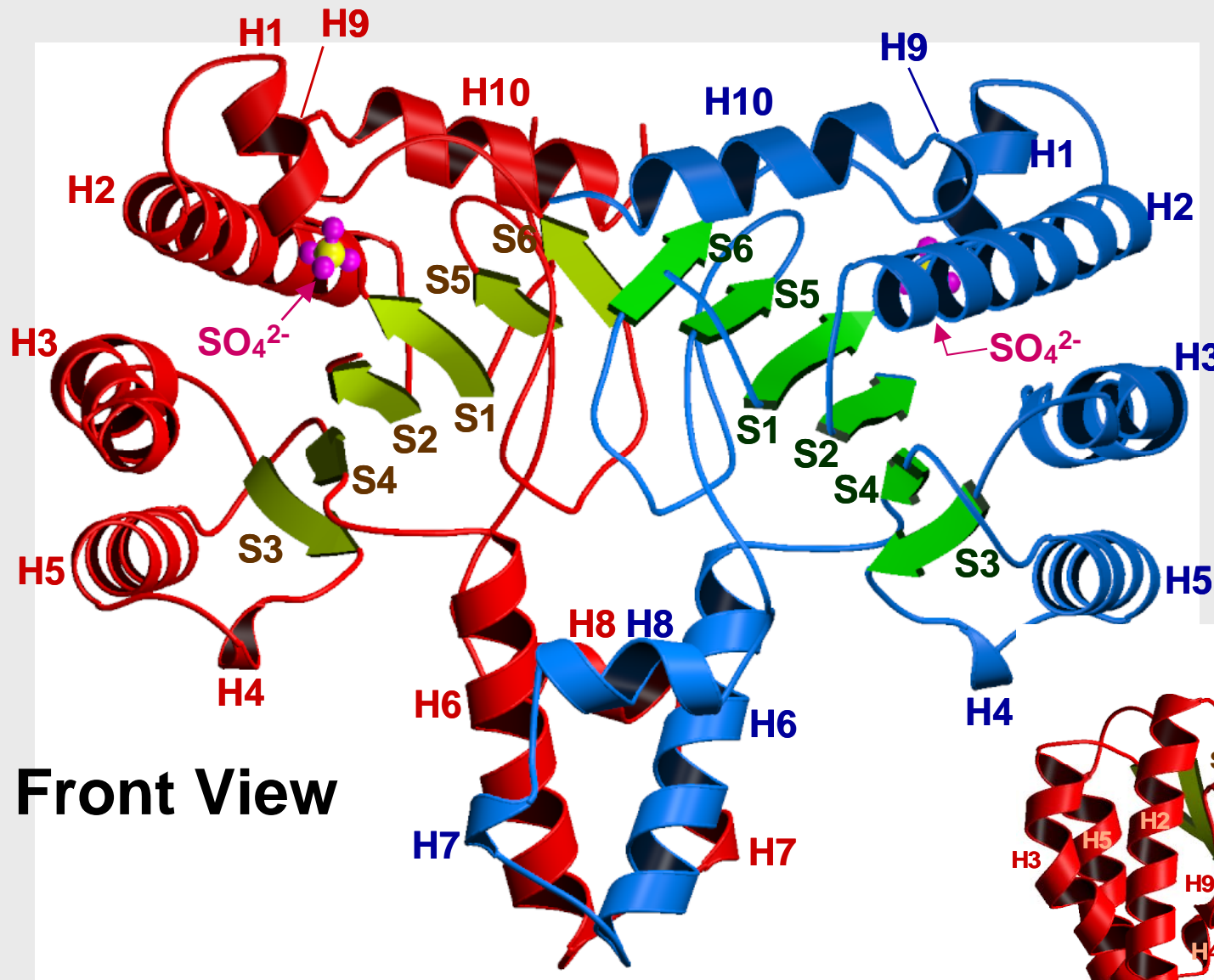


1.2 mm

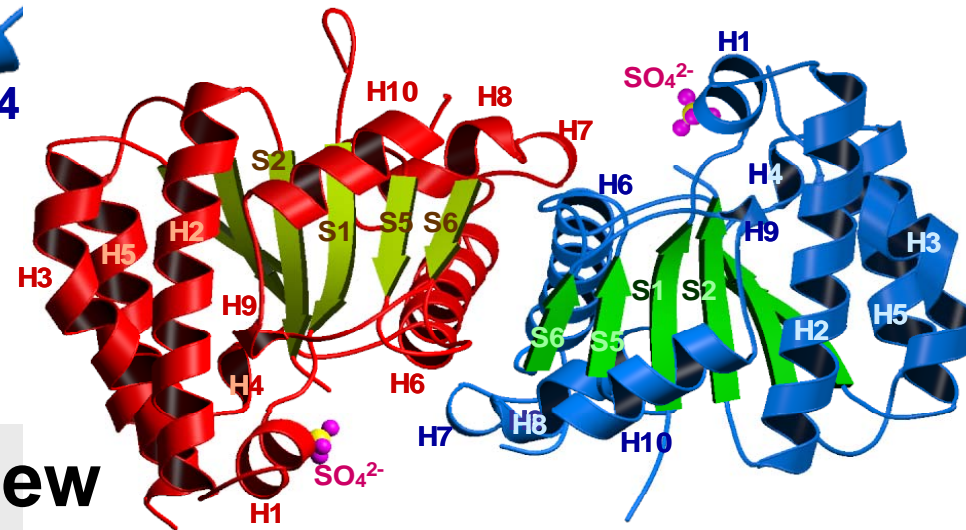
Resolution	2.2 Å
$R_{\text{merge}}$	3.2%
Completeness	83.7%

M. Fujihashi *et al.*, *Acta Crystallographica Section D*, 55, 1606-1607 (1999)

# Total Structure of *Micrococcus luteus* B-P 26 UPS



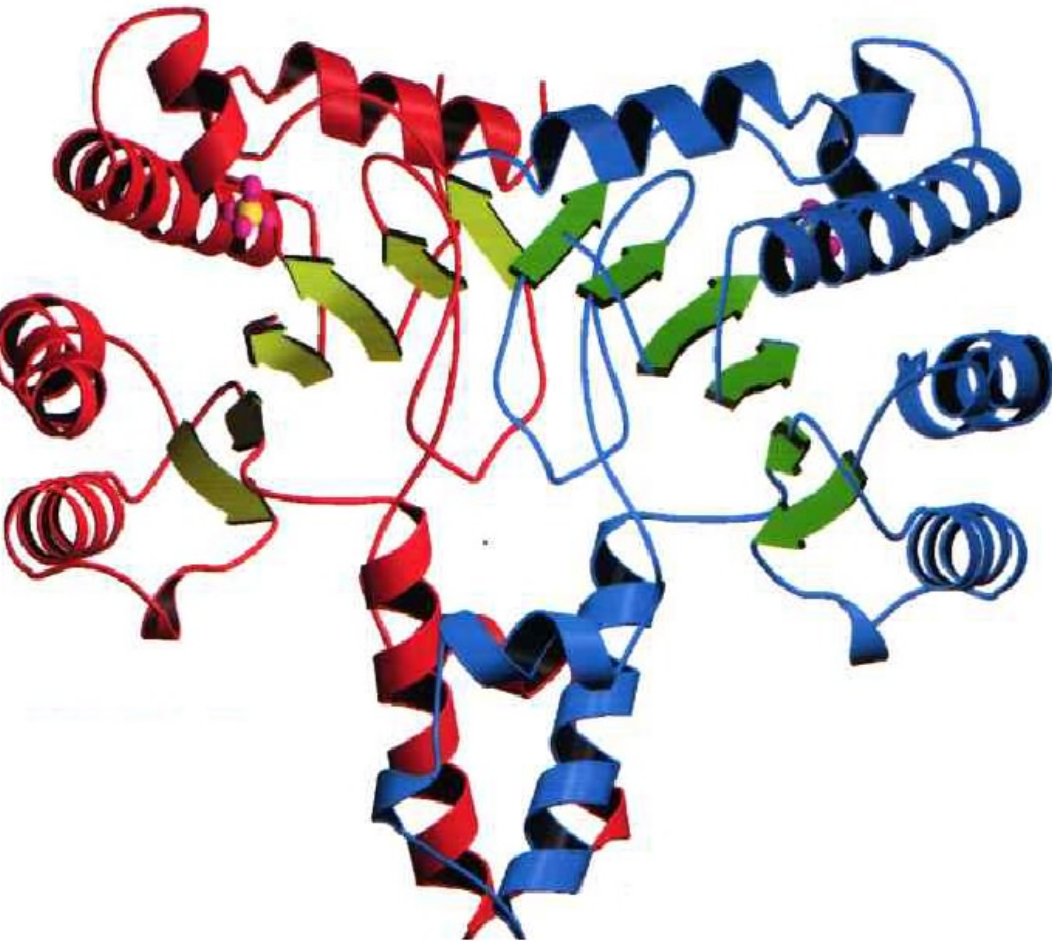
Top View



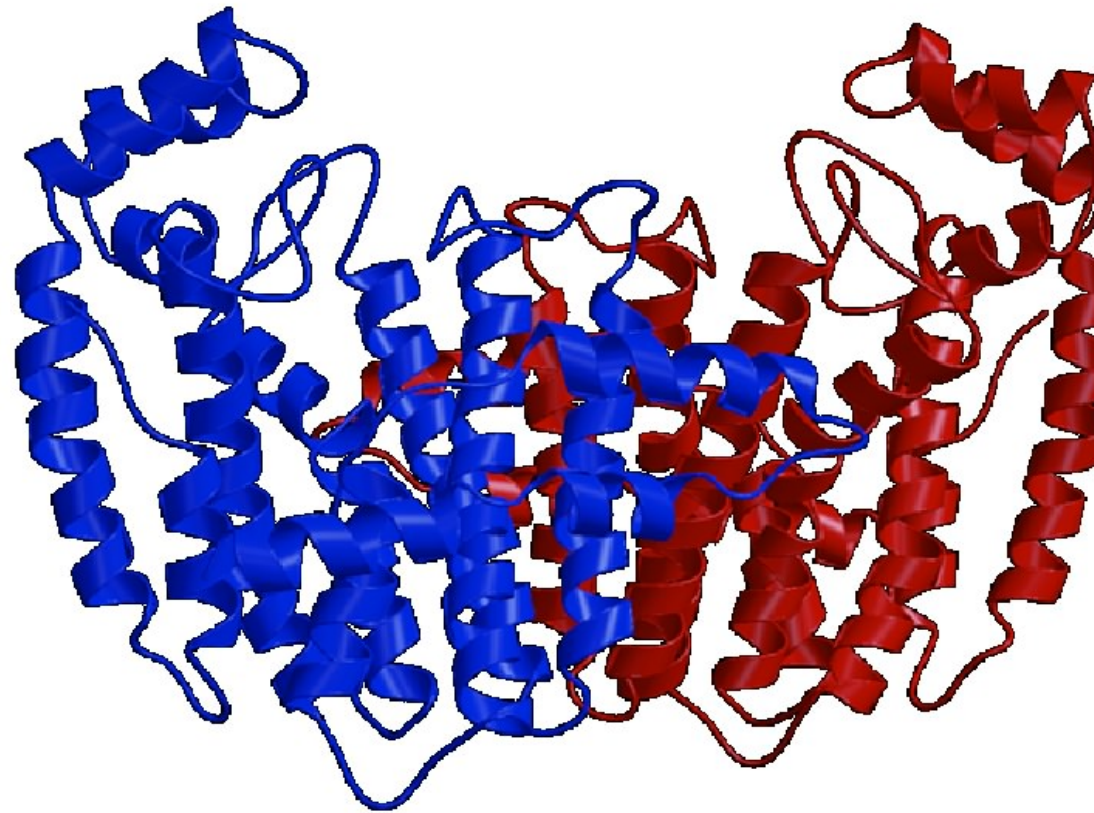


# Crystal structure of *Micrococcus luteus* B-P 26 undecaprenyl diphosphate synthase and Avian FPP synthase

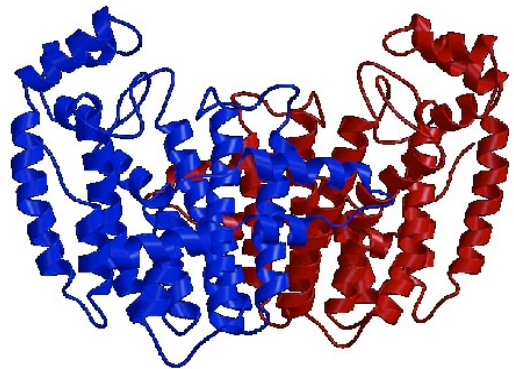
*Micrococcus luteus* B-P 26 UPS



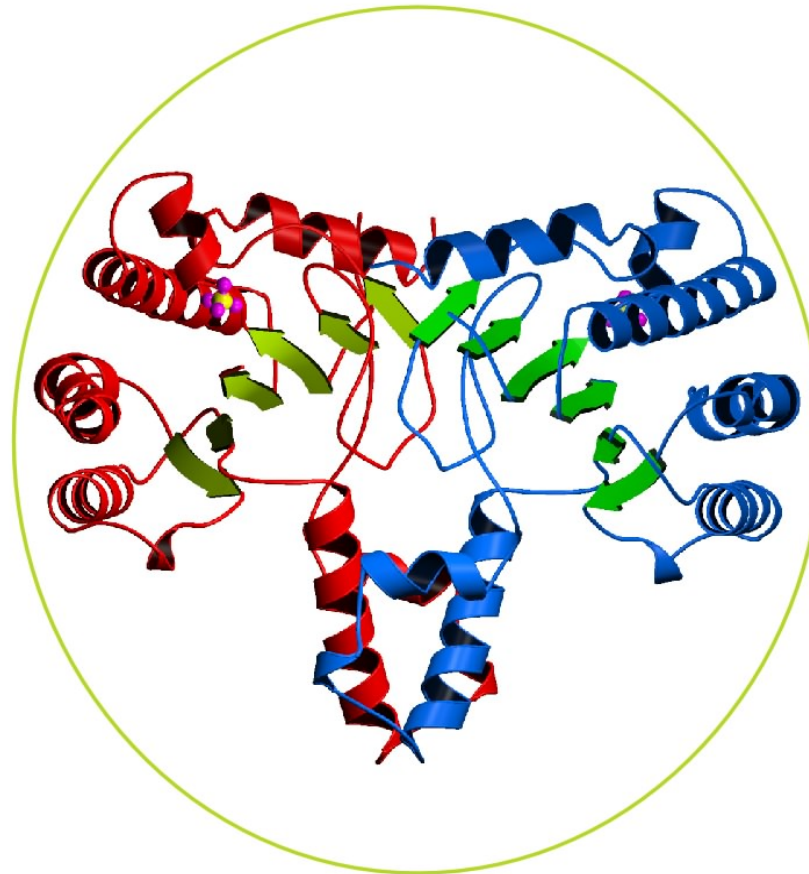
Avian Liver FPP Synthase



# The Structure of UPS is much different from the “Isoprenoid Synthase Fold”



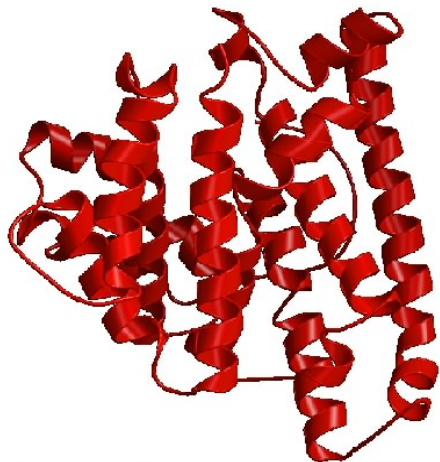
Farnesyl Diphosphate Synthase



UPS



5-Epi-Aristolochene Synthase

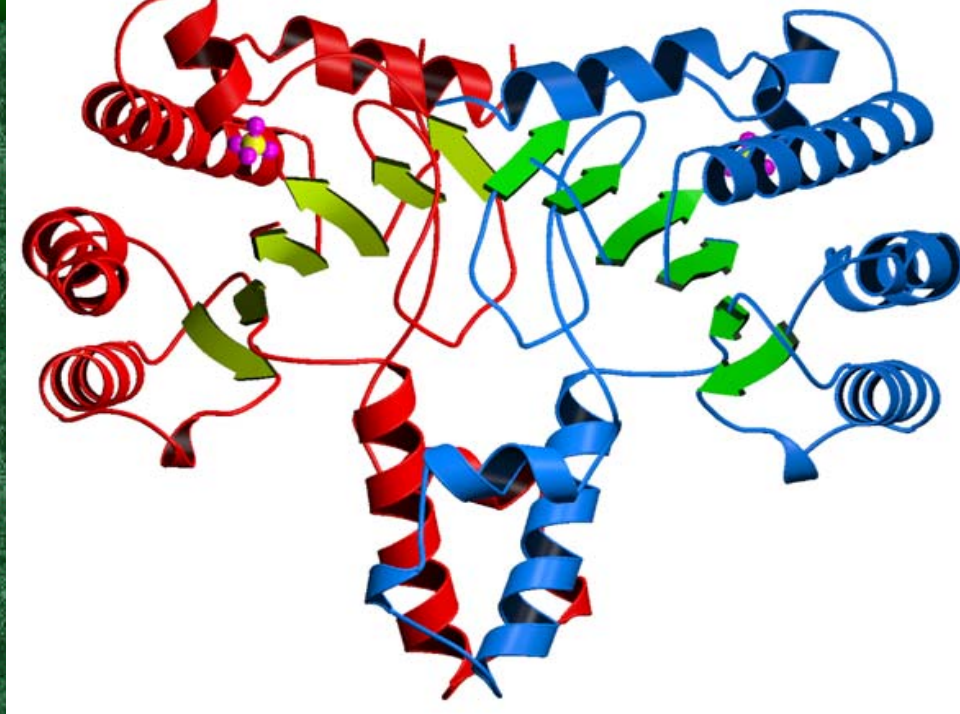


Pentalenene Synthase

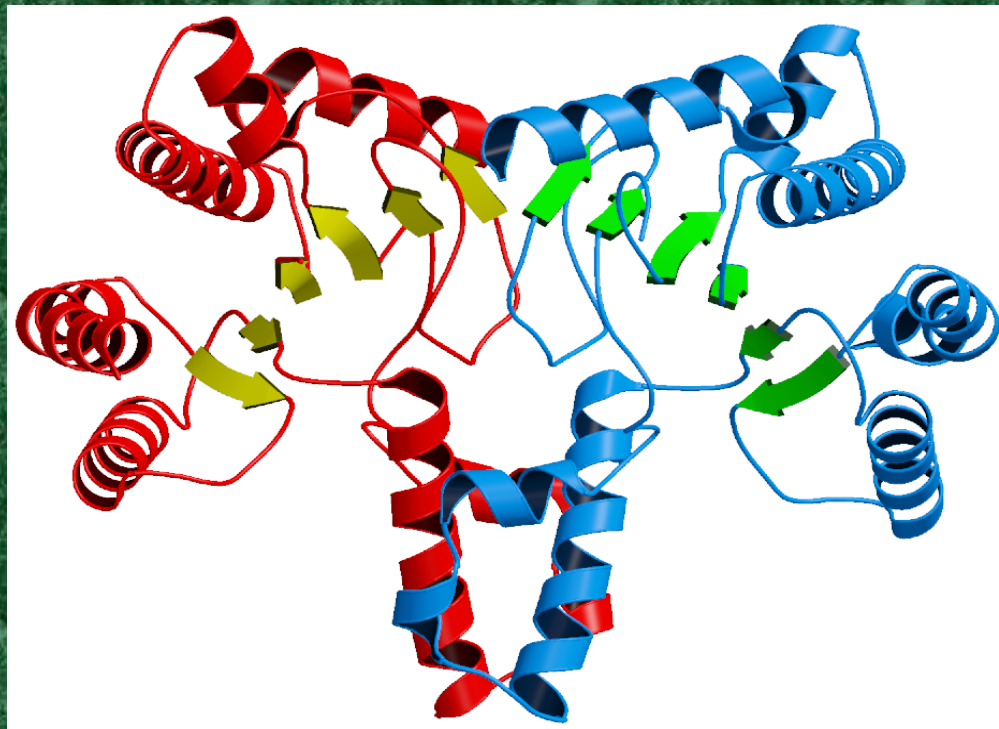


Squalene Cyclase





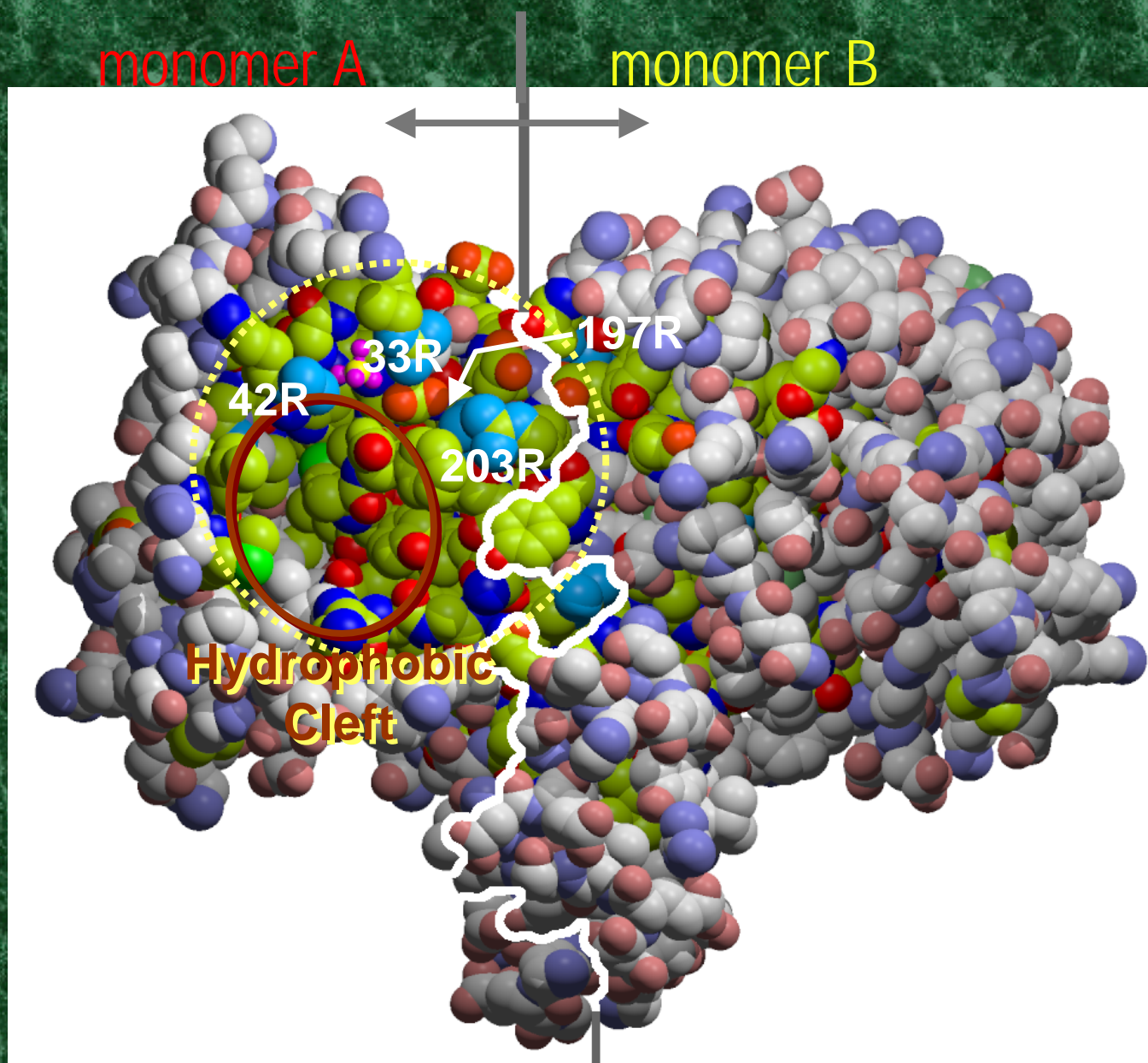
*M. luteus* UPS [Fujihashi *et al.*, *Proc. Natl. Acad. Sci., USA.*, **98**, 4337-4342 (2001)]



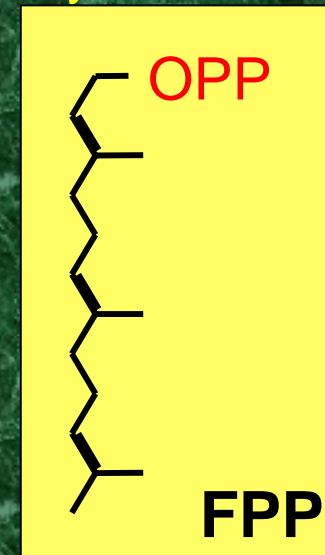
*E. coli* UPS [Kestell *et al.*, *J. Biol. Chem.*, **276**, 47474-47482 (2001)]



# Conserved Regions

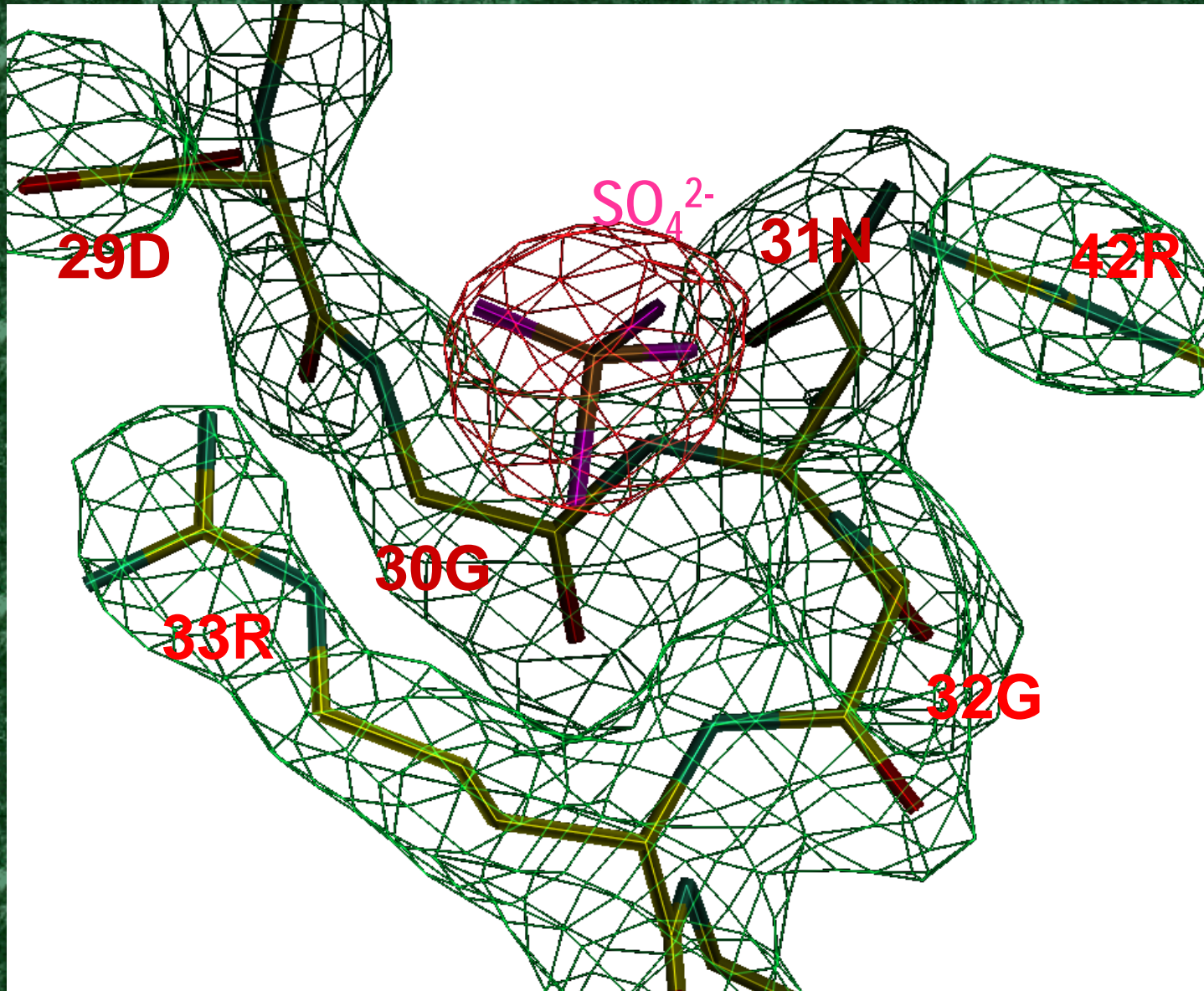


Allylic Substrate



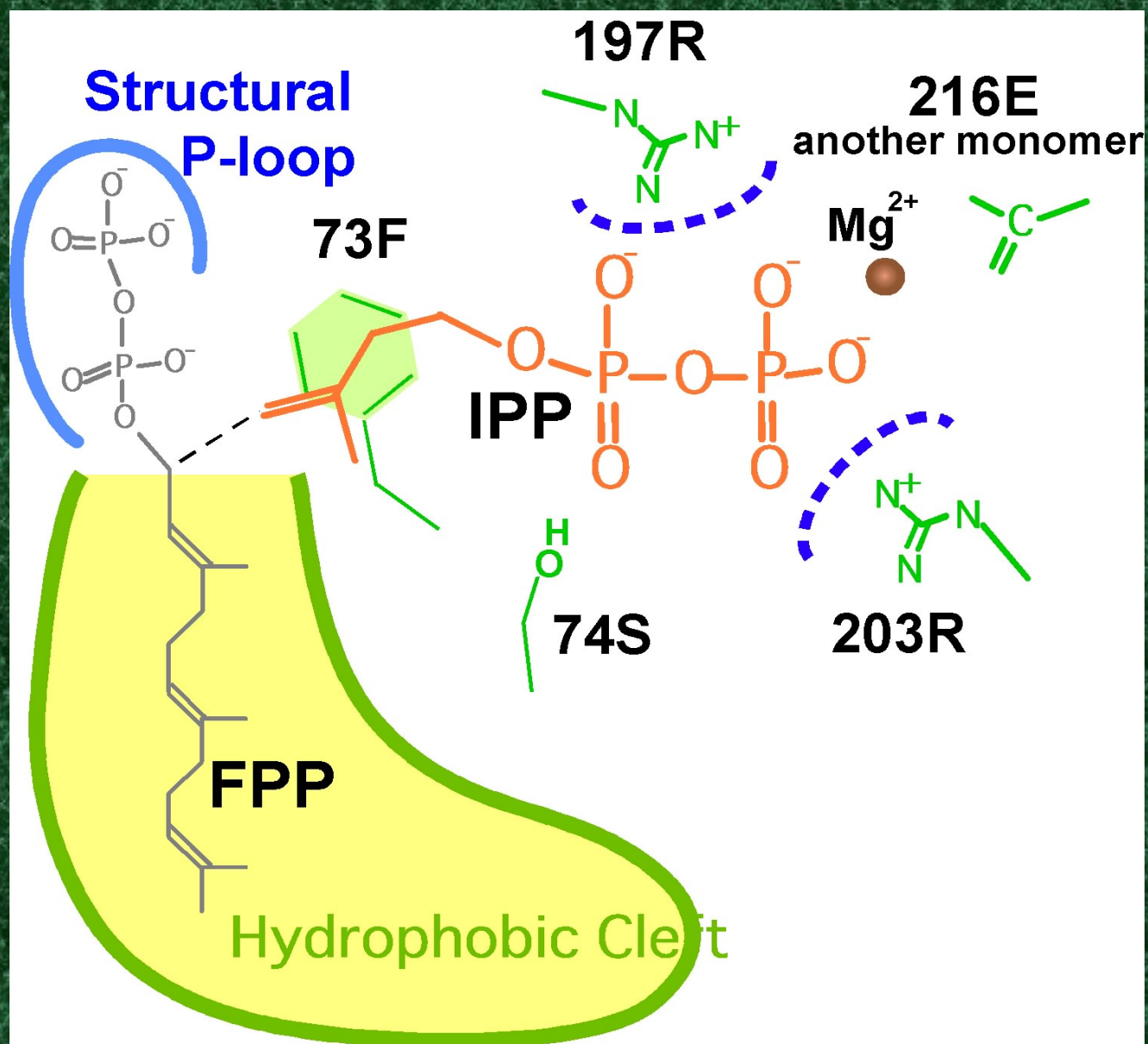


# A Sulfate Ion binds to the Structural P-loop



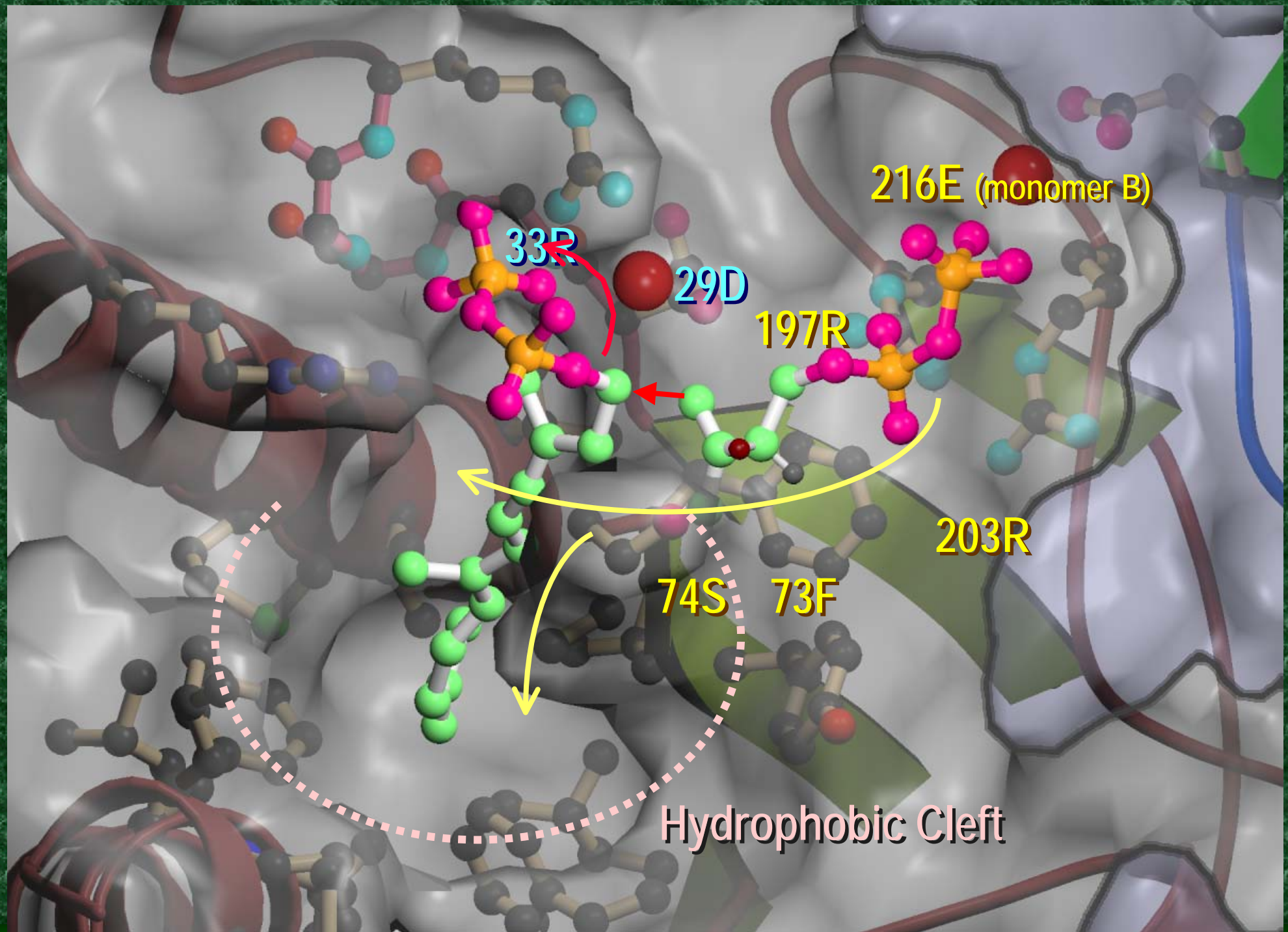


# Proposed Substrate Binding Model of the UPP Synthase





# A Probable Catalytic Reaction Model





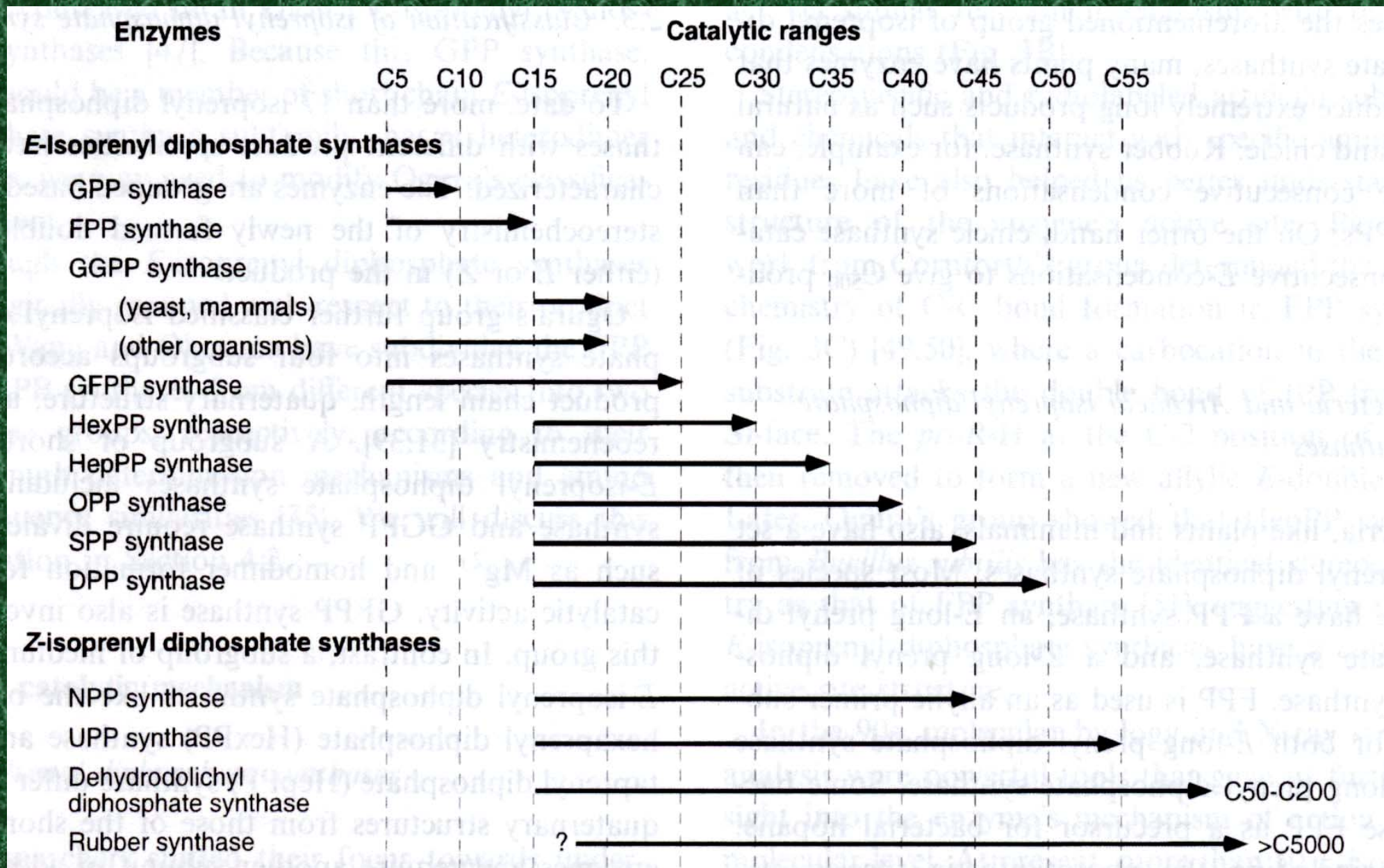
- # Chain Length Determination Mechanisms

1. *E*-Prenyltransferases

2. *Z*-Prenyltransferases



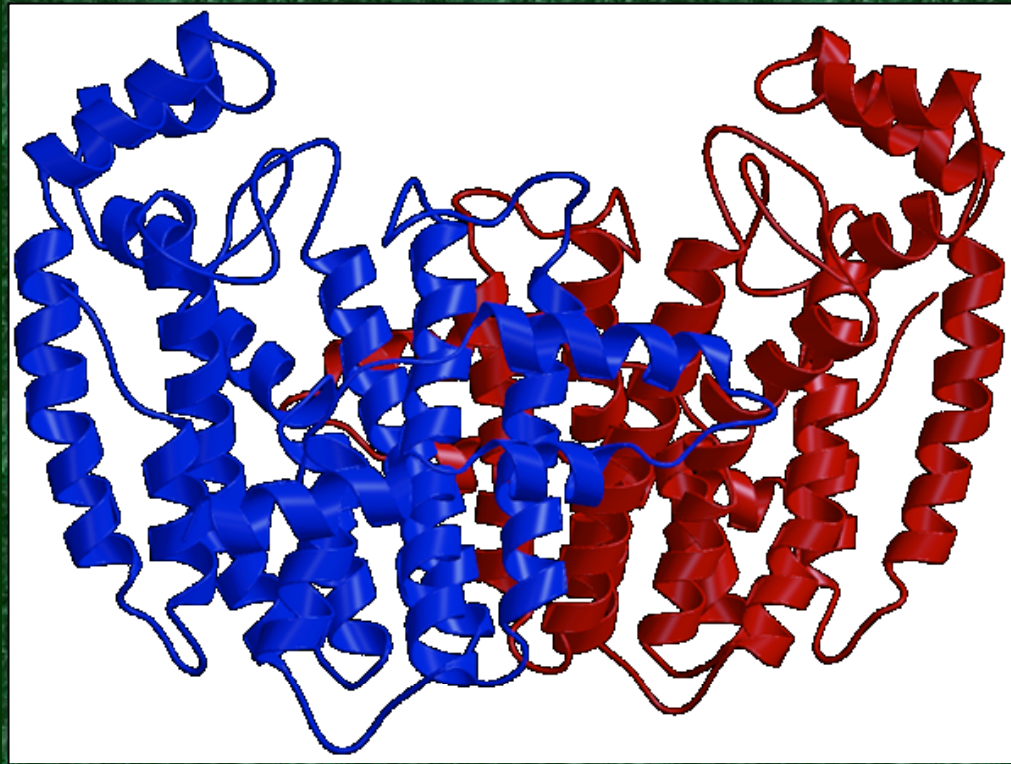
# Catalytic Ranges of Prenyl Chain Elongation by Prenyltransferases so far Characterized





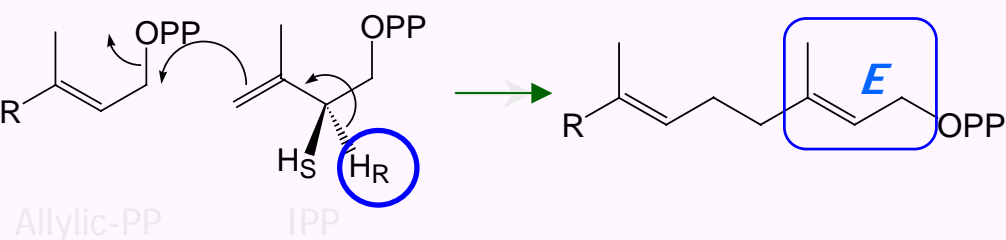
# Prenyltransferases

▪ *trans*-prenyltransferase

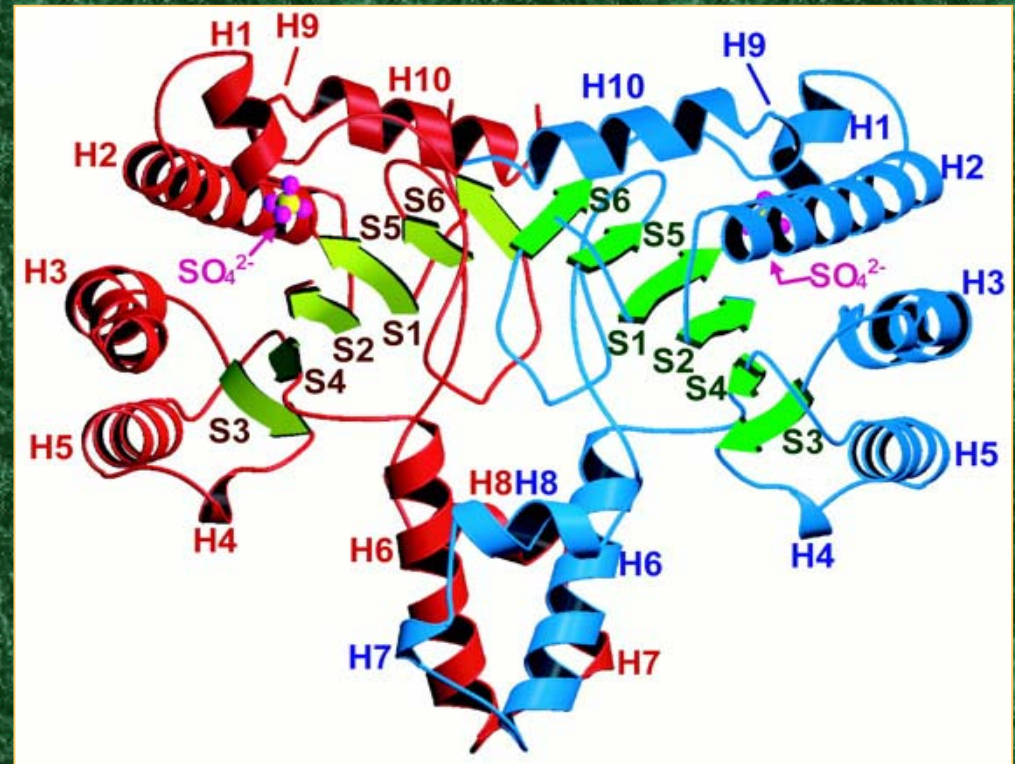


**Avian Liver FPP synthase**

Tarshis, L. C. *et al. Biochemistry*, 33, 10871-10877 (1994)

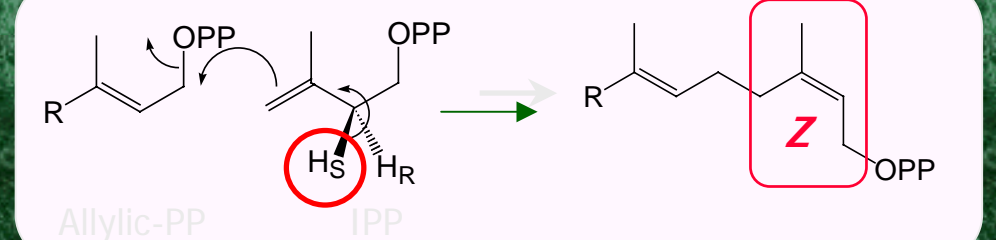


▪ *cis*-prenyltransferase



***Micrococcus luteus* B-P 26 UPP synthase**

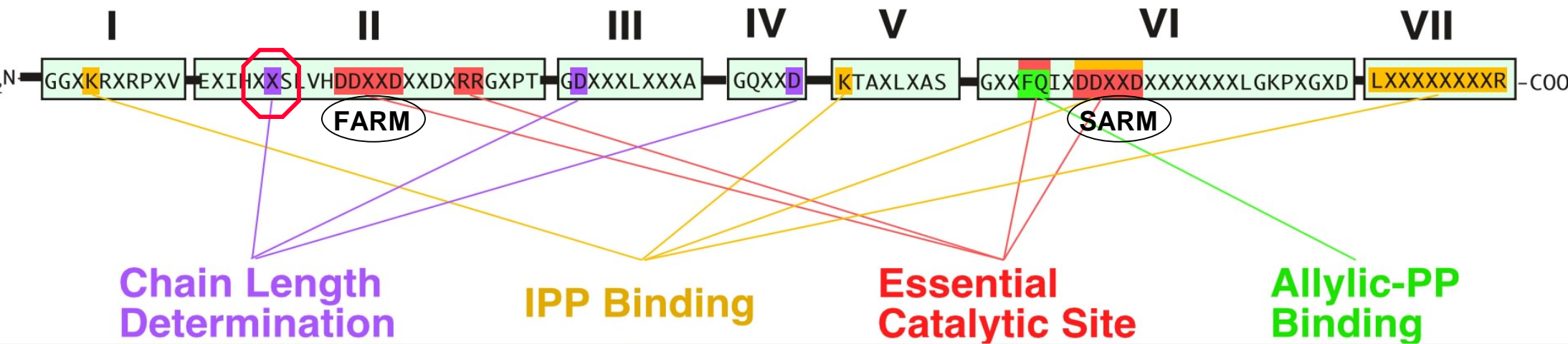
Fujihashi *et al. Proc Natl Acad Sci.* 4337-42 (2001)





# Roles of the Conserved Regions of *trans*-Prenyl Chain Elongating Enzymes

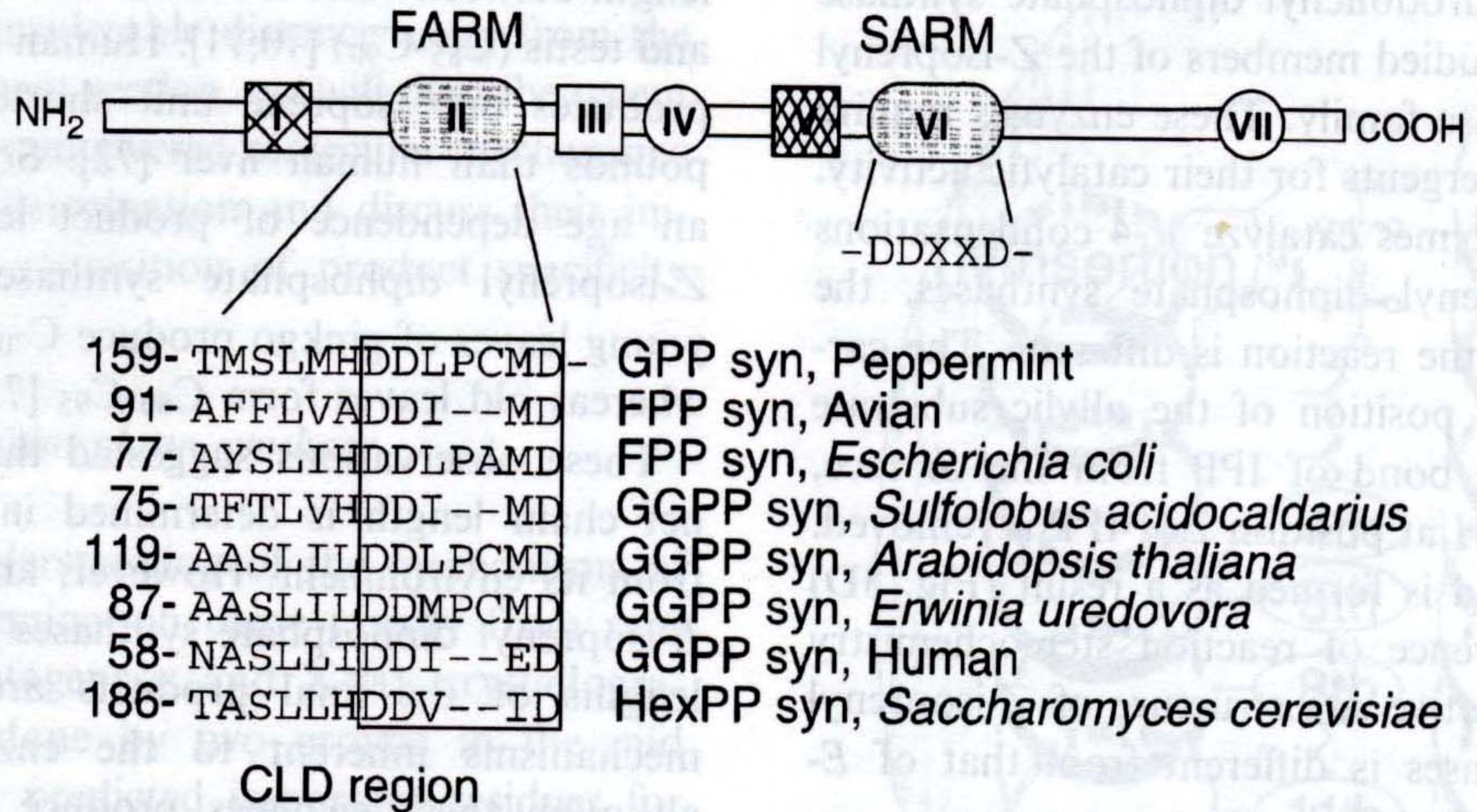
## *E*-Prenyl Diphosphate Synthases



Koyama, T. et al., Biochemistry, 35, 9533-9538 (1996); Koyama, T. et al., Biochem. Biophys. Res. Commun., 212, 681-686 (1995).; Koyama, T. et al., Can. J. Chem., 72, 75-79 (1994); Koyama, T. et al., Biochemistry, 33, 12644-12648 (1994); Ohnuma, S. et al., J. Biol. Chem., 271, 18831-18837 (1997); Ohnuma, S. et al., J. Biol. Chem., 272, 5192-5198 (1997)

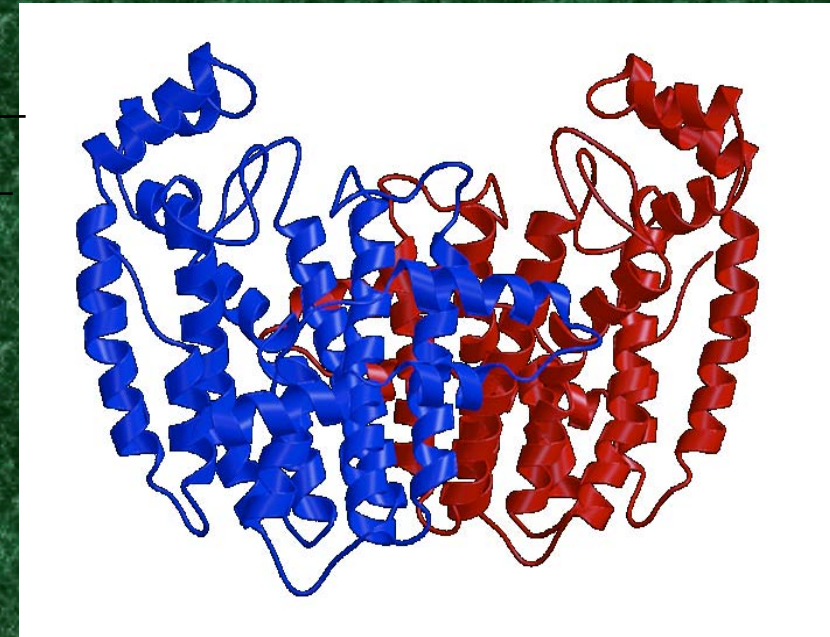
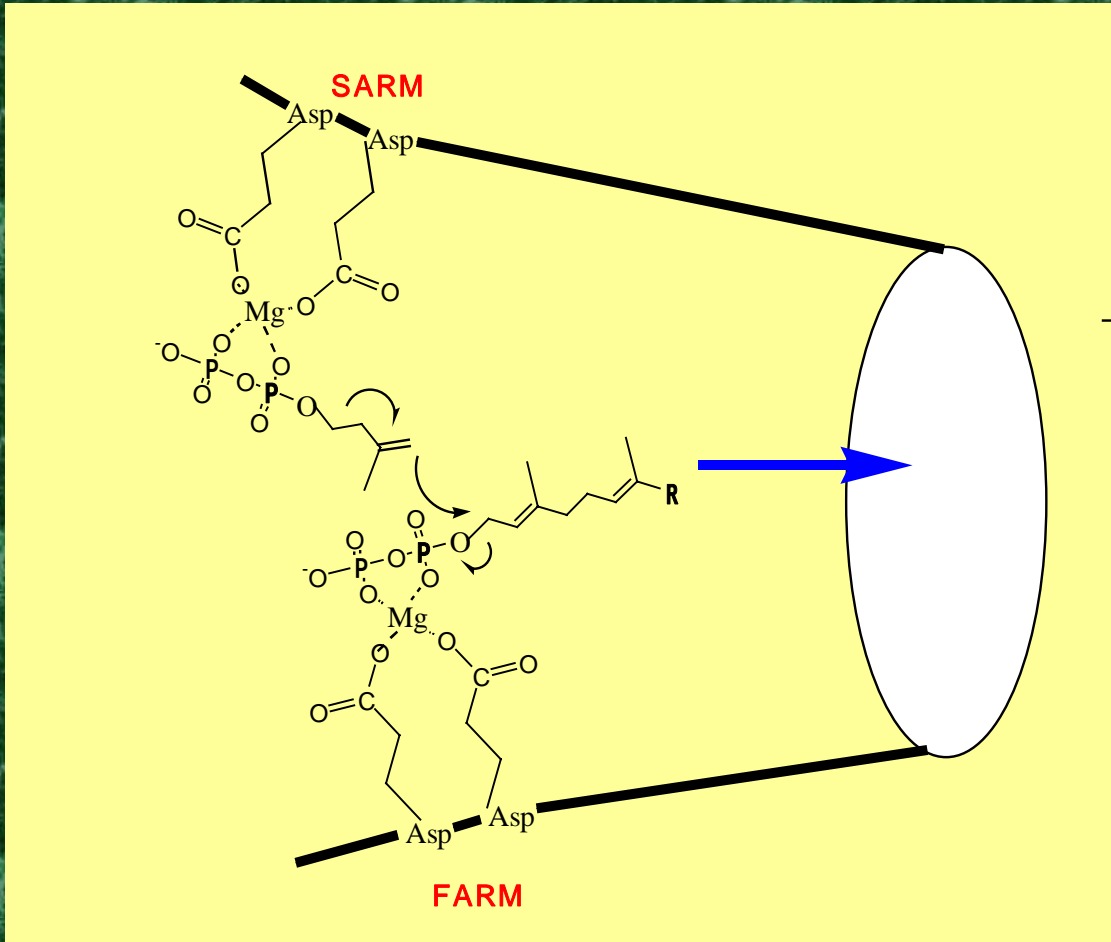
First Aspartate-Rich Motif

Second Aspartate-Rich Motif

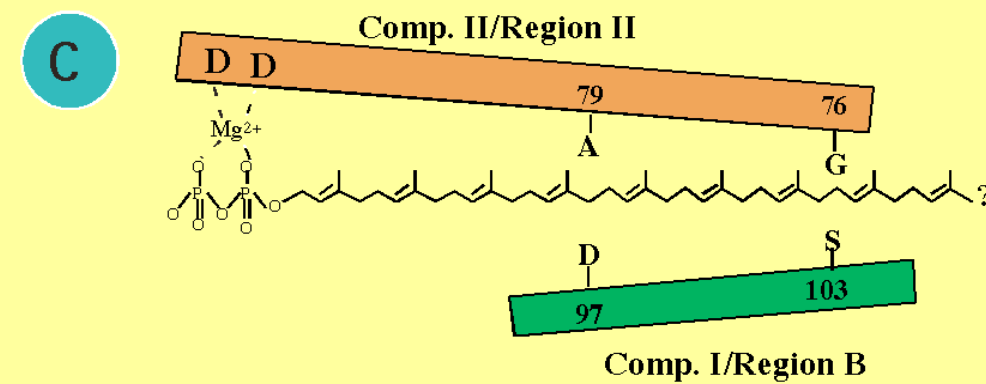
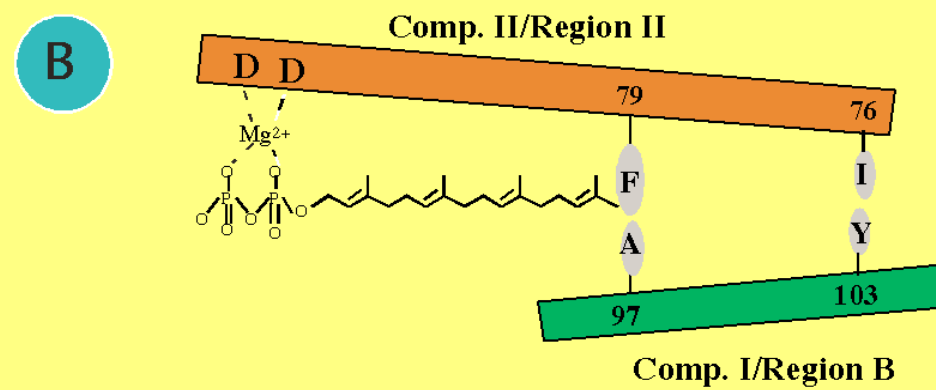
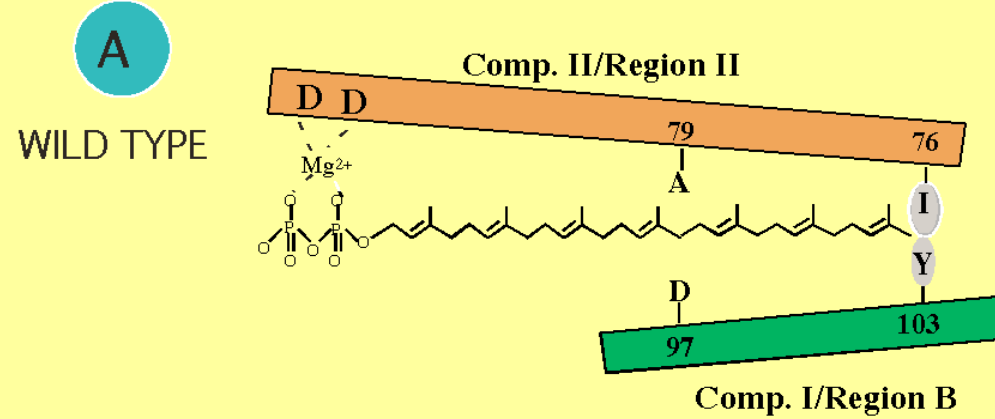




# Product Chain Length Determination Mechanism of *trans*-Prenyltransferase



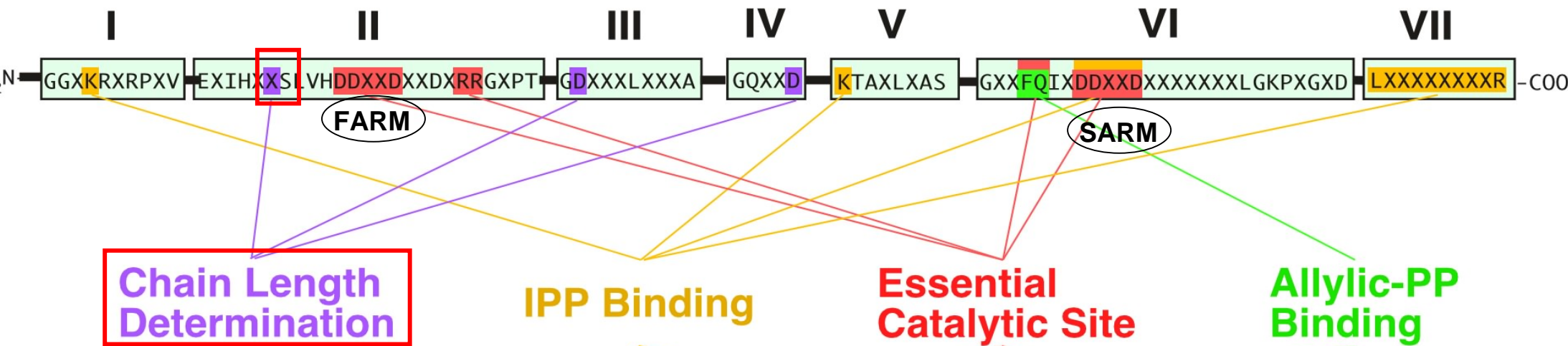
**S. Ohnuma *et al.*, *J. Biol. Chem.*, 271, 10087-10095 (1996).;**  
**L. C. Tarshis *et al.*, *Proc. Natl. Acad. Sci., USA*, 93, 15018-15023 (1996).;**  
**S. Ohnuma *et al.*, *J. Biol. Chem.*, 271, 30748-30754 (1996).**



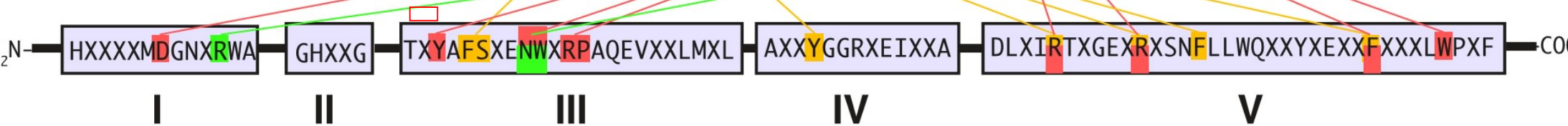


# Comparison of Conserved Regions of *E*- and *Z*-Prenyl Diphosphate Synthases

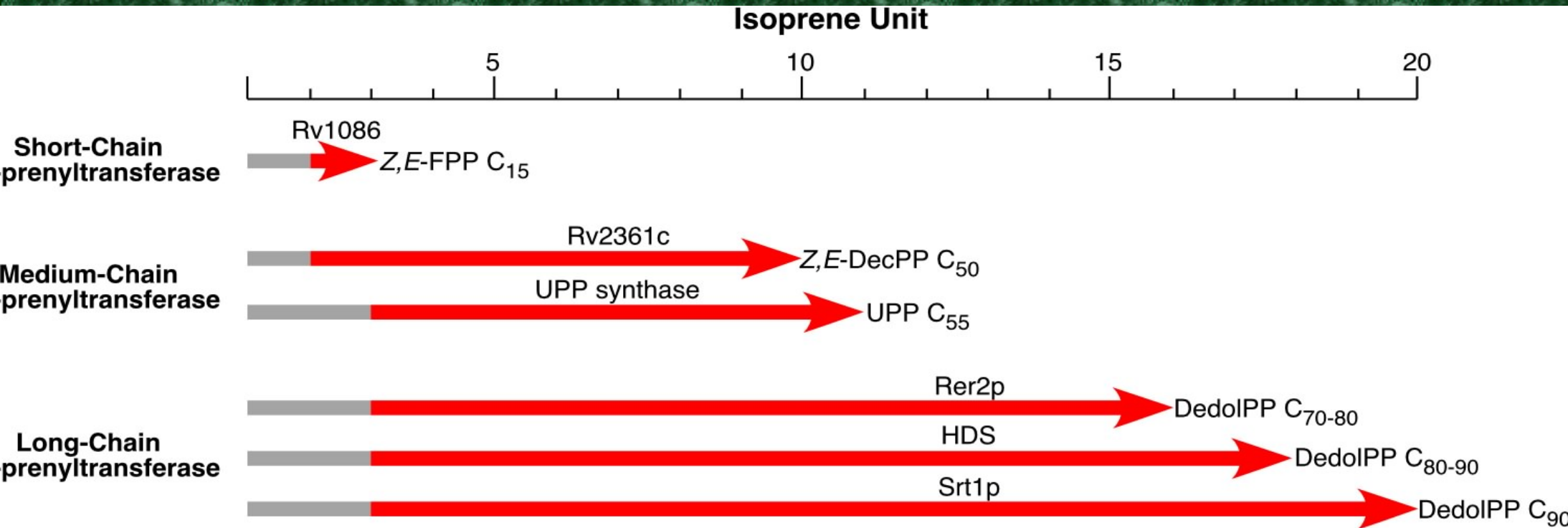
## *E*-Prenyl Diphosphate Synthases



## *Z*-Prenyl Diphosphate Synthases

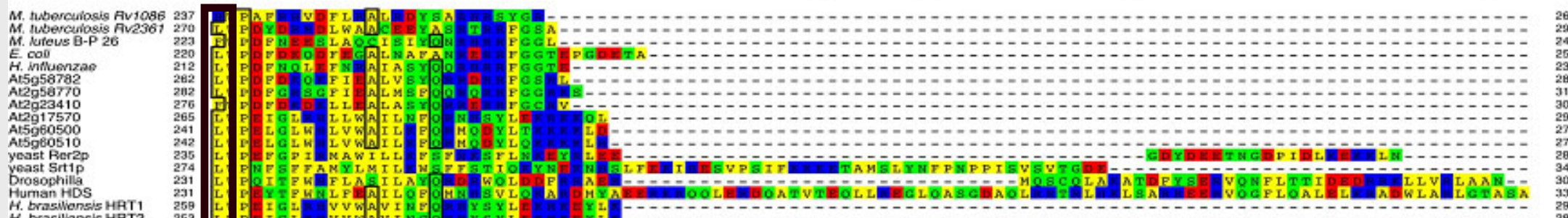
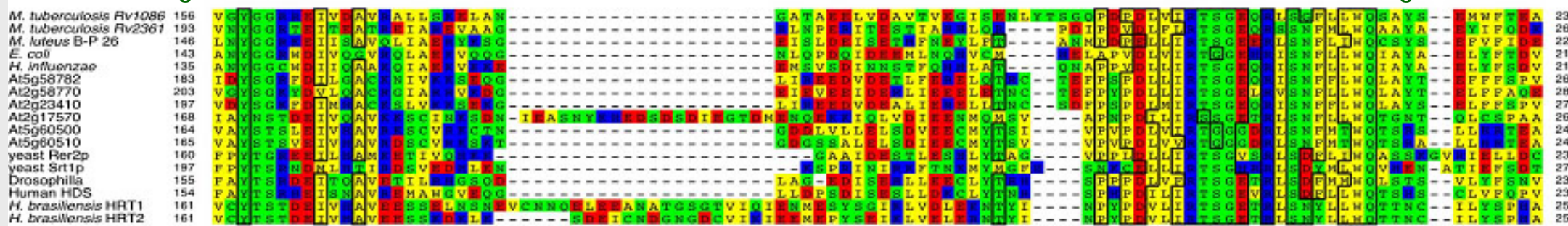
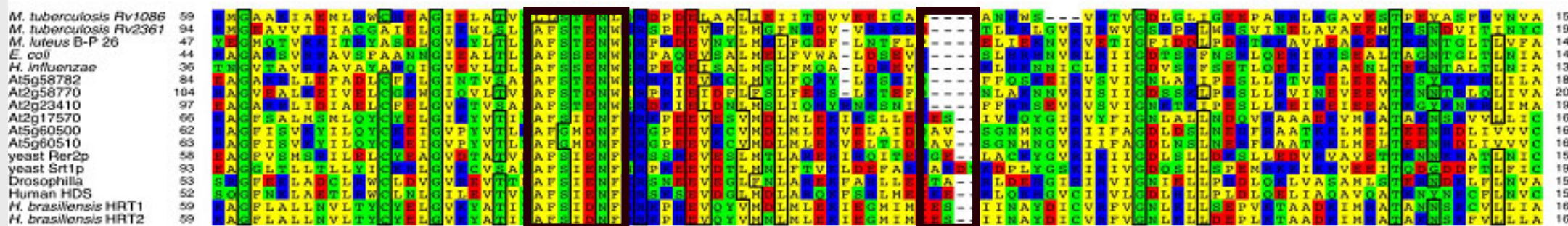
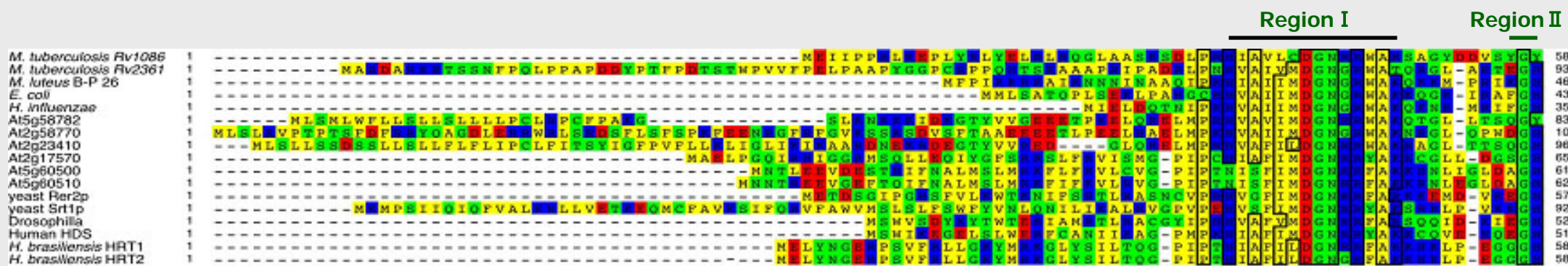


# Classification of *cis*-Prenyltransferases





# Amino Acid Alignments of *cis*-Prenyltransferases





# Characteristic Regions in Short *cis*-Prenyltransferase

**Region III**

Rv1086	80	A	T	V	Y	L	L	S	T	E	N	L	Q	R	D	P	D	E	L	A	98
Rv2361	115	L	S	L	Y	A	F	S	T	E	N	W	K	R	S	P	E	E	V	R	134
<i>M. luteus</i>	68	L	T	L	Y	A	F	S	T	E	N	W	S	R	P	K	D	E	V	N	86
<i>E. coli</i>	65	L	T	L	Y	A	F	S	S	E	N	W	N	R	P	A	Q	E	V	S	83
Rer2p	79	A	T	V	F	A	F	S	I	E	N	F	K	R	S	S	R	E	V	E	97
HDS	74	V	T	V	Y	A	F	S	I	E	N	F	K	R	S	K	S	E	V	D	92
Srt1p	114	V	S	A	Y	A	F	S	I	E	N	F	N	R	P	K	E	E	V	D	132

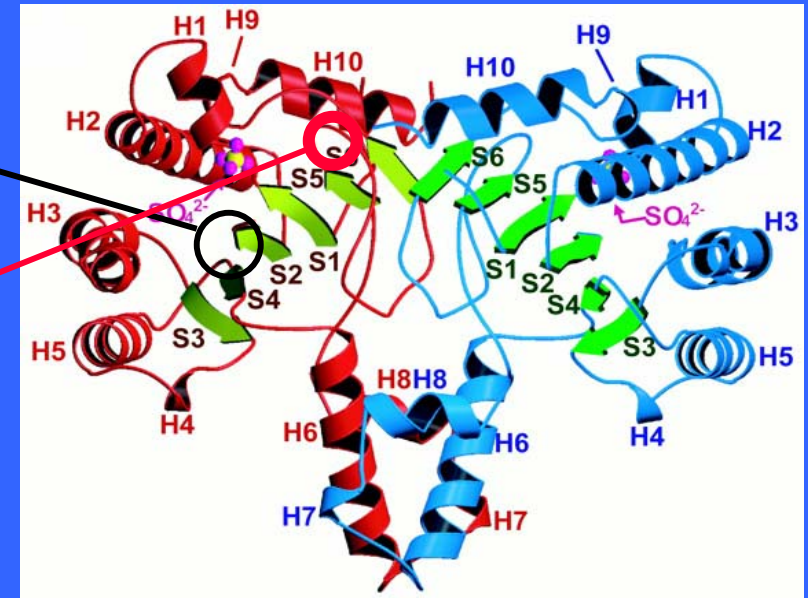
Sheet-2      Flexible domain

### Region V

Rv1086	224	W	Q	S	A	Y	S	-	-	E	M	W	F	T	E	A	H	W	P	A	F	241
Rv2361	257	W	Q	A	A	Y	A	-	-	E	Y	I	F	Q	D	K	L	W	P	D	Y	274
M. luteus	210	W	Q	C	S	Y	S	-	-	E	F	V	F	I	D	E	F	W	P	D	F	227
E. coli	207	W	Q	I	A	Y	A	-	-	E	L	Y	F	T	D	V	L	W	P	D	F	224
Rer2p	219	W	Q	A	S	S	K	G	V	R	I	E	L	L	D	C	L	W	P	E	F	241
HDS	218	W	Q	T	S	H	S	-	-	C	L	V	F	Q	P	V	L	W	P	E	Y	235
Srt1p	259	W	Q	V	H	E	N	-	A	T	I	E	F	S	D	T	L	W	P	N	F	278

Sheet-6

Helix-9



*M. luteus* B-P 26 UPP synthase  
Fujihashi et al. Proc. Natl. Acad. Sci. 4337-42 (2001)

- *cis*-FPS WT      82- VYLLSTENLQR –92
- AF (L84A / L85F)      82- LYAFSTENLQR –92
- L84A      82- LYALSTENLQR –92
- L85F      82- LYL<sup>F</sup>STENLQR –92
- H237L      235- EALWP –239



# Product Analysis of UPS Mutant Enzymes

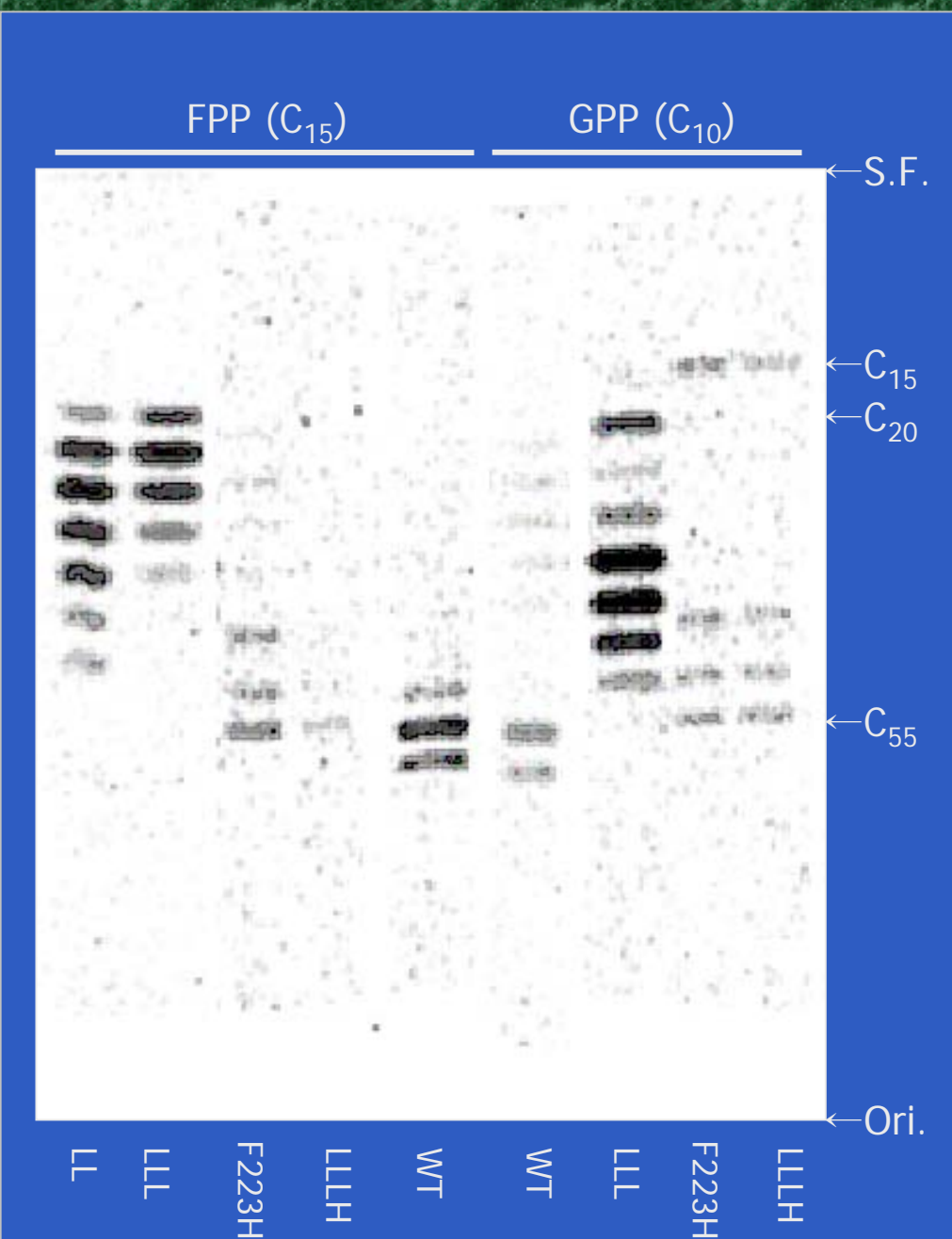
## *M. luteus* UPS mutant

Reversed phase TLC

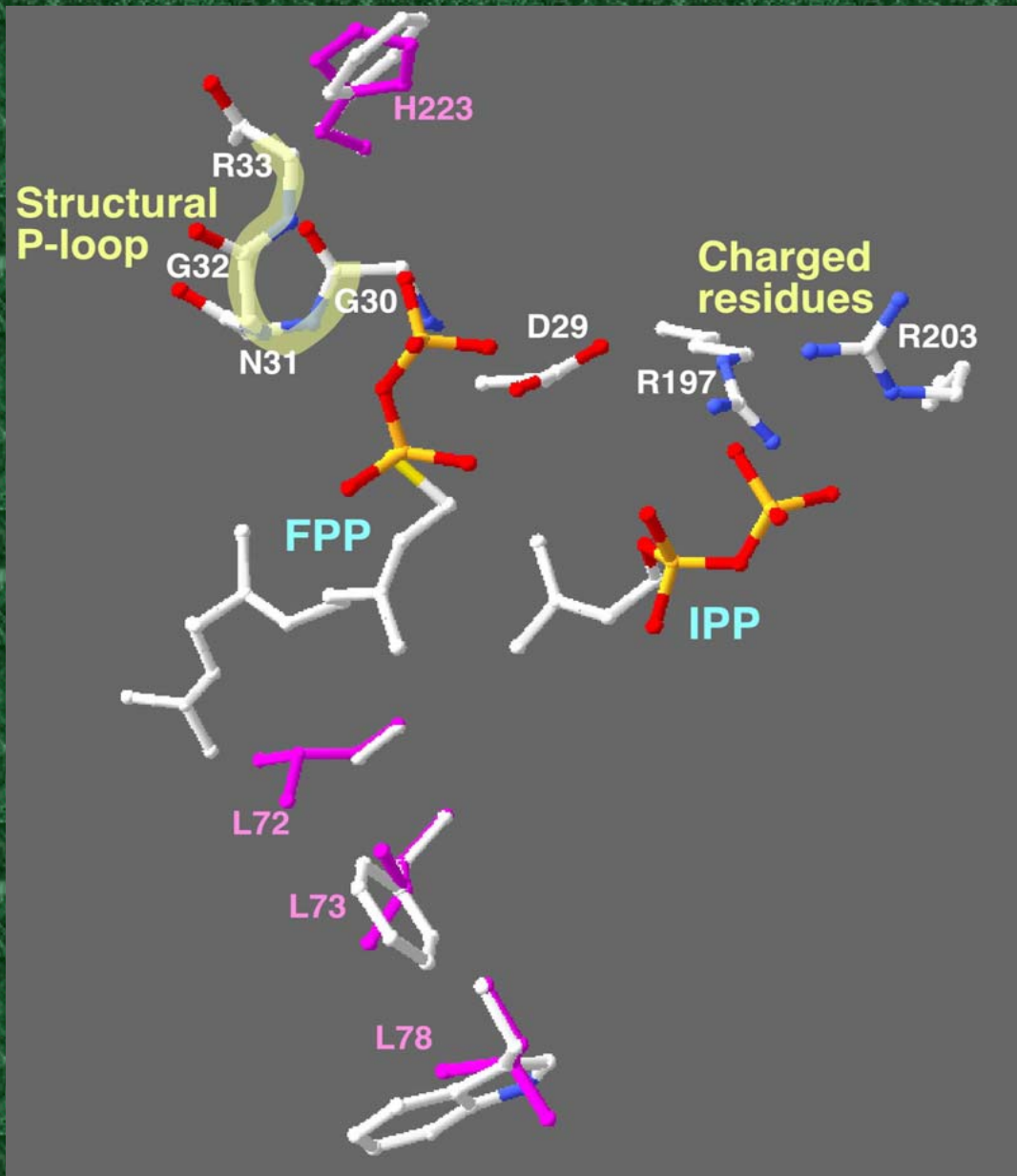
solvent ··· Acetone/water(19:1)

- |          |                     |                 |
|----------|---------------------|-----------------|
| • UPS WT | 70- LYAFSTENWSR -80 | 221- DEFWP -225 |
| • LL     | 70- LYLLSTENWSR -80 |                 |
| • LLL    | 70- LYLLSTENLSR -80 |                 |
| • F223H  |                     | 221- DEHWP -225 |
| • LLLH   | 70- LYLLSTENLSR -80 | 221- DEHWP -225 |

Kharel, Y. et al., *FEBS J.*, 273, 647-657 (2006)



# Structure Model of the LLLH Mutant of UPS



- Leu-84

Ala-72 in *M. luteus* UPS

...close to the  $\omega$ -end carbon of FPP

⇒Side-chain of Leu may **interfere** with elongation of product.

Chang, S.Y. *et al.*, *Protein Sci.*, 13, 971 (2004)



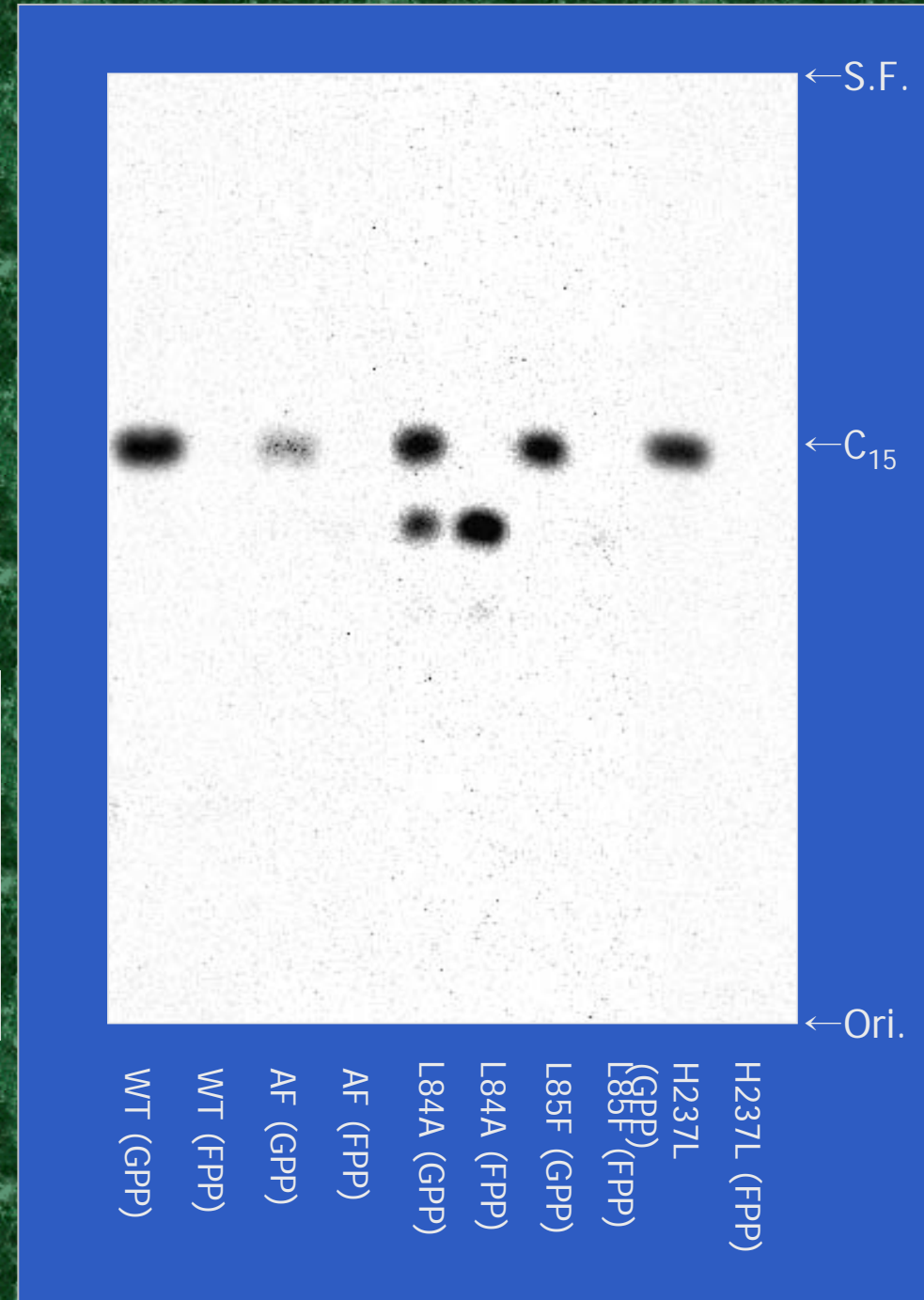
# Product Analysis of FPS Mutant Enzymes

## *M. tuberculosis* FPS mutant

## Reversed phase TLC

solvent... MeOH/Acetone(8:2)

- *cis*-FPS WT 82- VYLLSTENLQR –92 265- EAHWP -239
- AF 82- LYAFSTENLQR –92
- L84A 82- LYALSTENLQR –92
- L85F 82- LYL~~F~~STENLQR –92
- H237L 265- EALWP -239



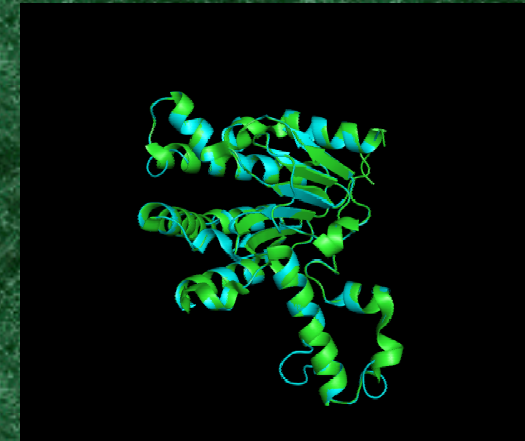
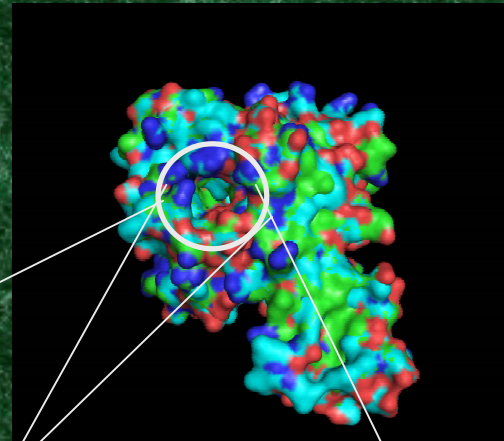
Noike, M. et al., *Biochem. Biophys. Res. Commun.*,  
377, 17-22 (2008)



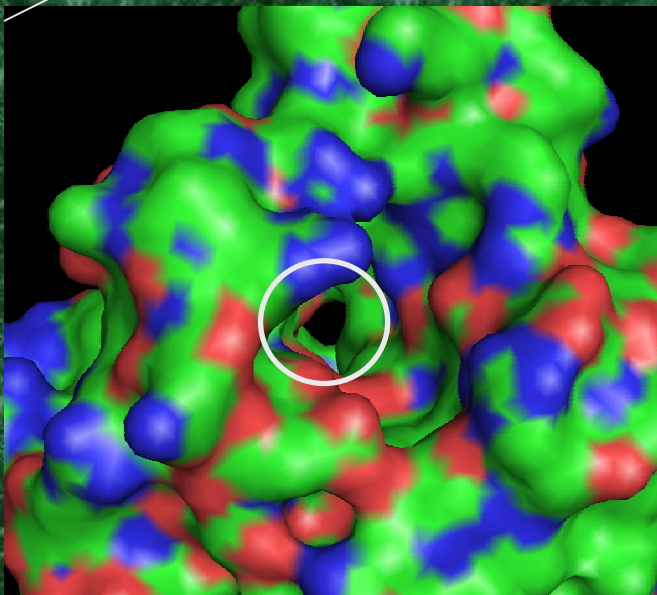
# 分子モデリング

SWISS-MODEL programを使用し、*Thermobifida fusca cis*-FPSの一次配列からモデルを作成

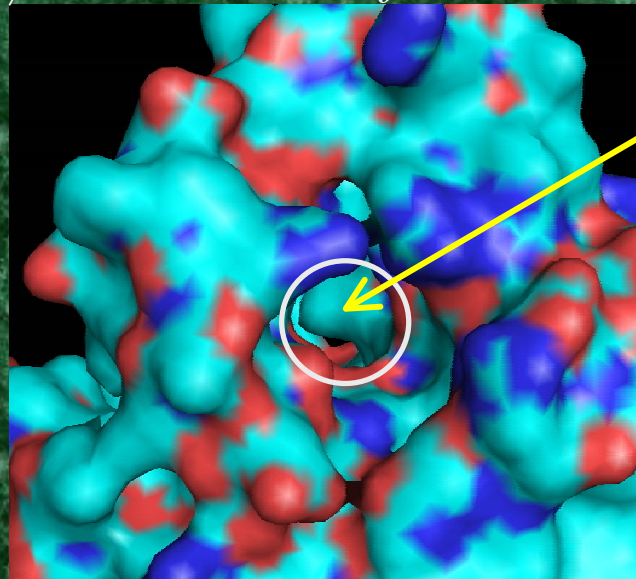
テンプレート：  
*E. coli* UPS FPP結合型構造  
(PDB ID : 1v7u) Chang *et al.* (2004)



*E. coli* UPS



*T. fusca* FPS

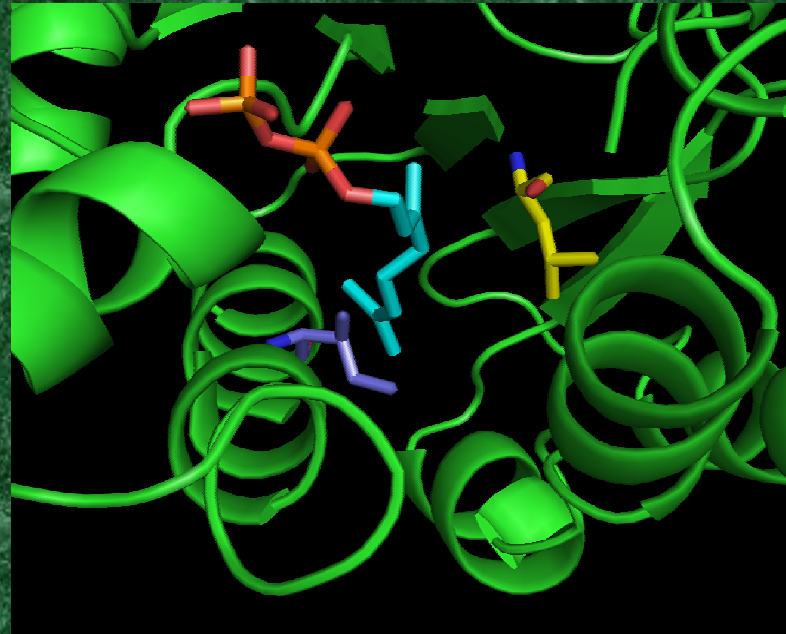
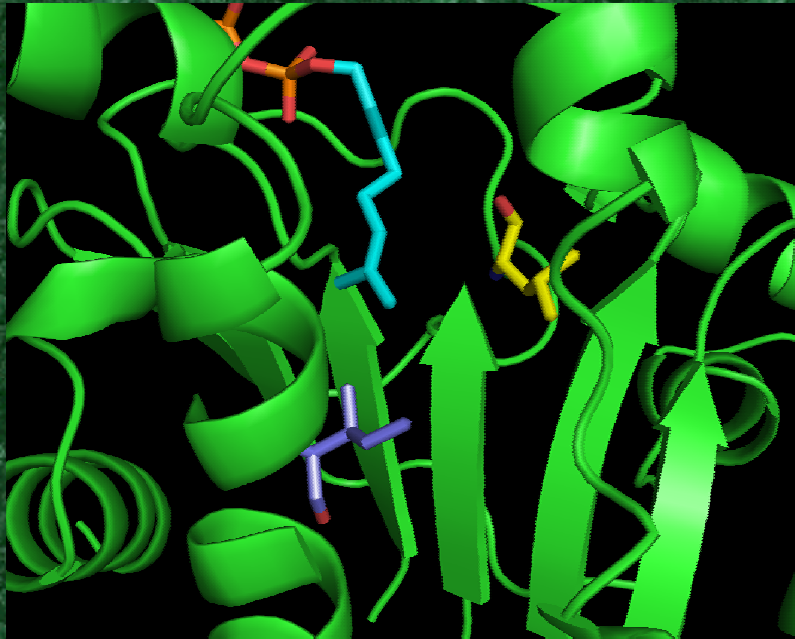





Ile-59



# *T. fusca cis*-FPS の基質結合型モデル

## *T. fusca cis*-FPSの基質結合型モデル



 : GPP  
 : Ile59  
 : Leu78

## Ile残基の周辺の部分アライメント

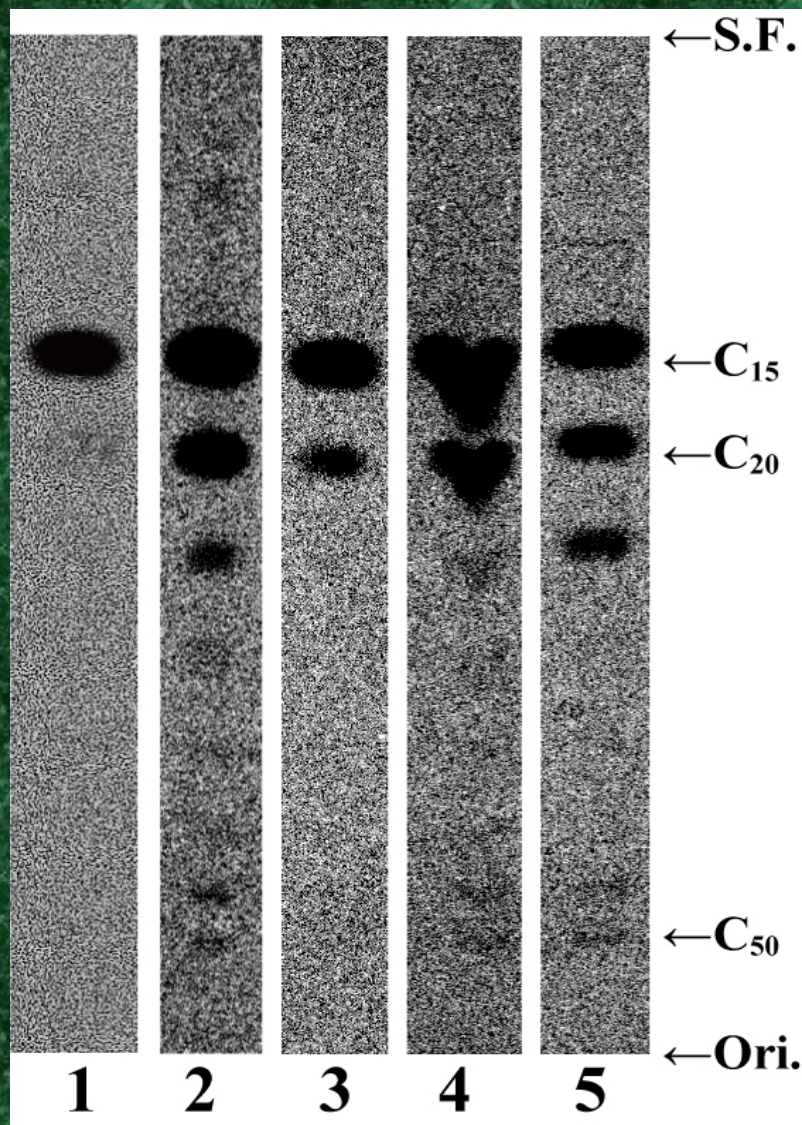
Cg_FPS	55	G	A	K	K	I	G	E	M	V	63
Ce_FPS	103	G	A	K	K	I	G	E	L	V	111
Tf_FPS	55	G	A	E	K	I	F	E	L	L	63
Mt_FPS	61	G	A	A	K	I	A	E	M	L	69
Mt_DecPS	96	G	E	A	V	V	I	D	I	A	104
MI_UPS	49	G	M	Q	T	V	K	K	I	T	57
Ec_UPS	46	G	A	K	S	V	R	R	A	V	54
Hp_UPS	44	G	V	K	T	L	K	D	I	T	52
Hs_DedolPS	54	G	F	N	K	L	A	E	T	L	62
Sc_rer2	60	G	F	V	S	M	S	R	I	L	68
Sc_srt1	95	G	G	L	T	L	L	T	L	L	103



# 変異体の生成物鎖長解析

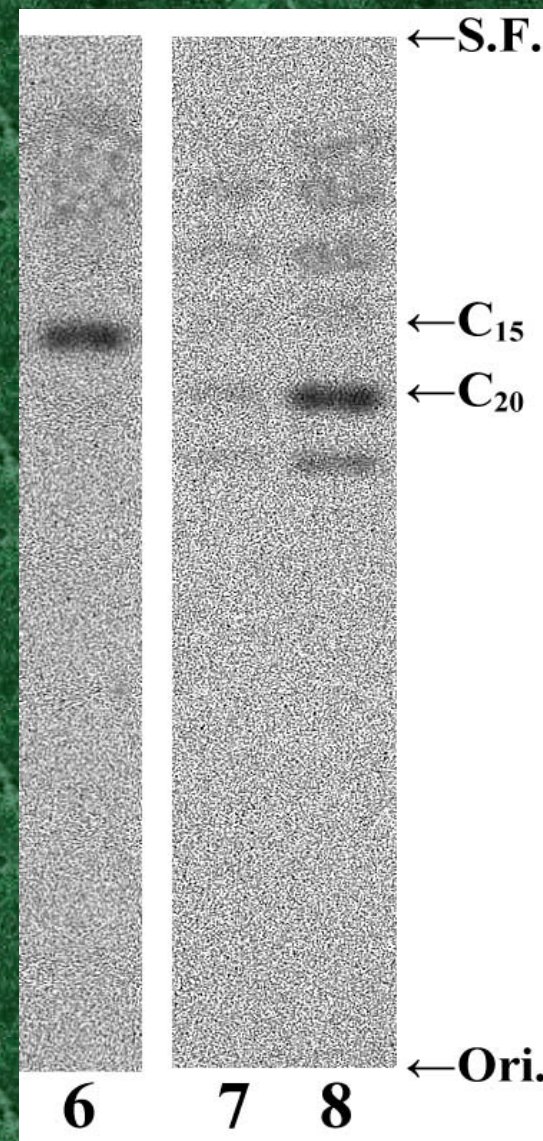
Ile59変異体

L78A変異体



展開溶媒

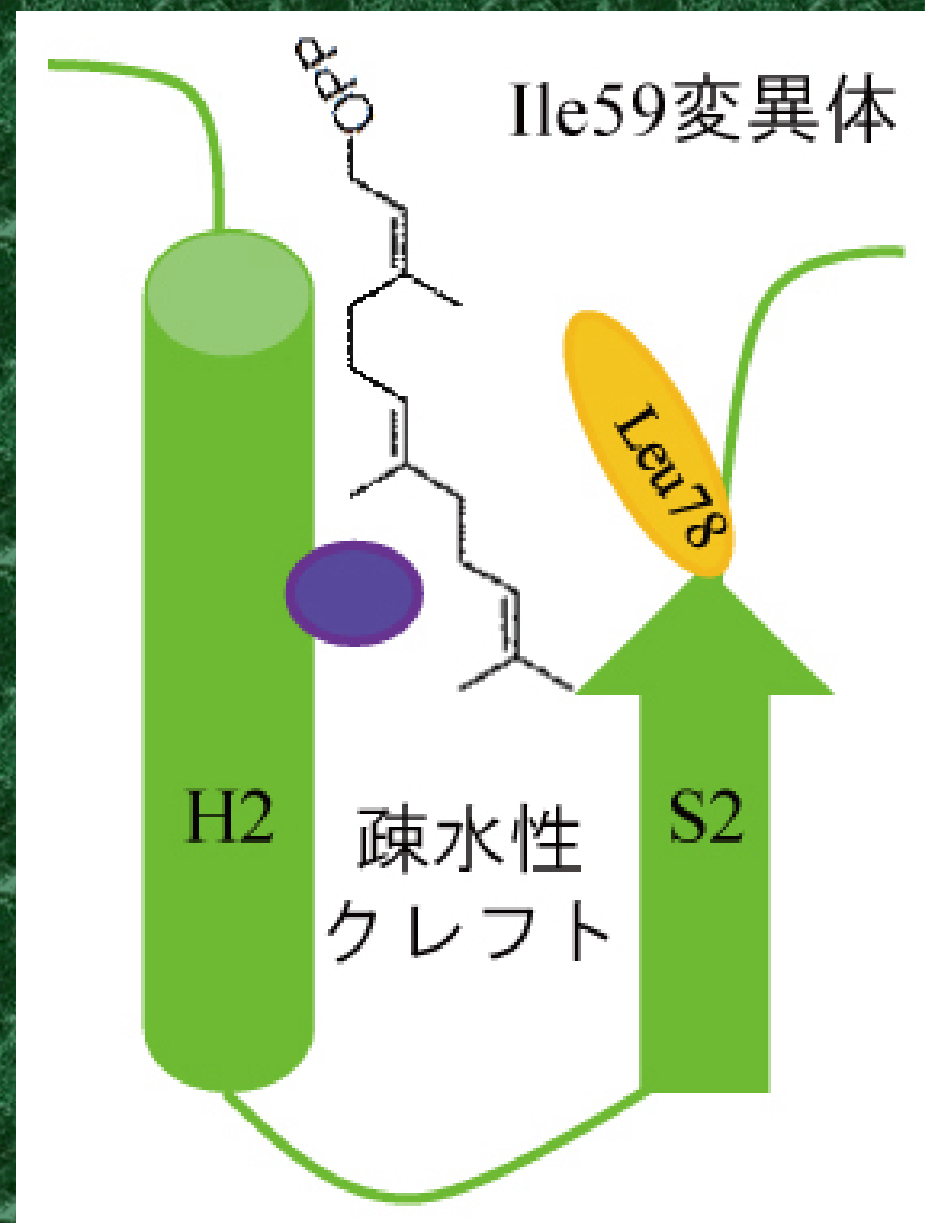
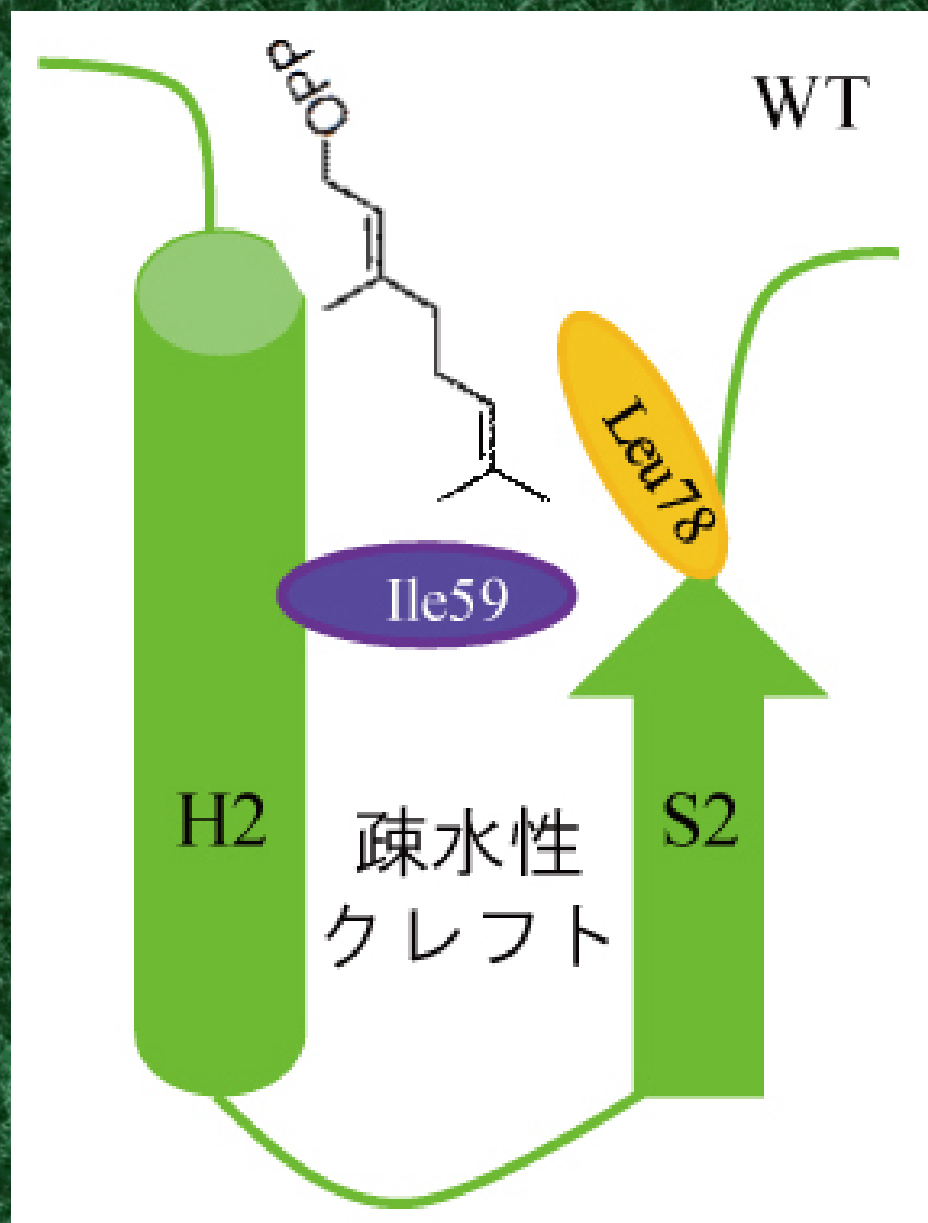
MeOH:Acetone=8:2

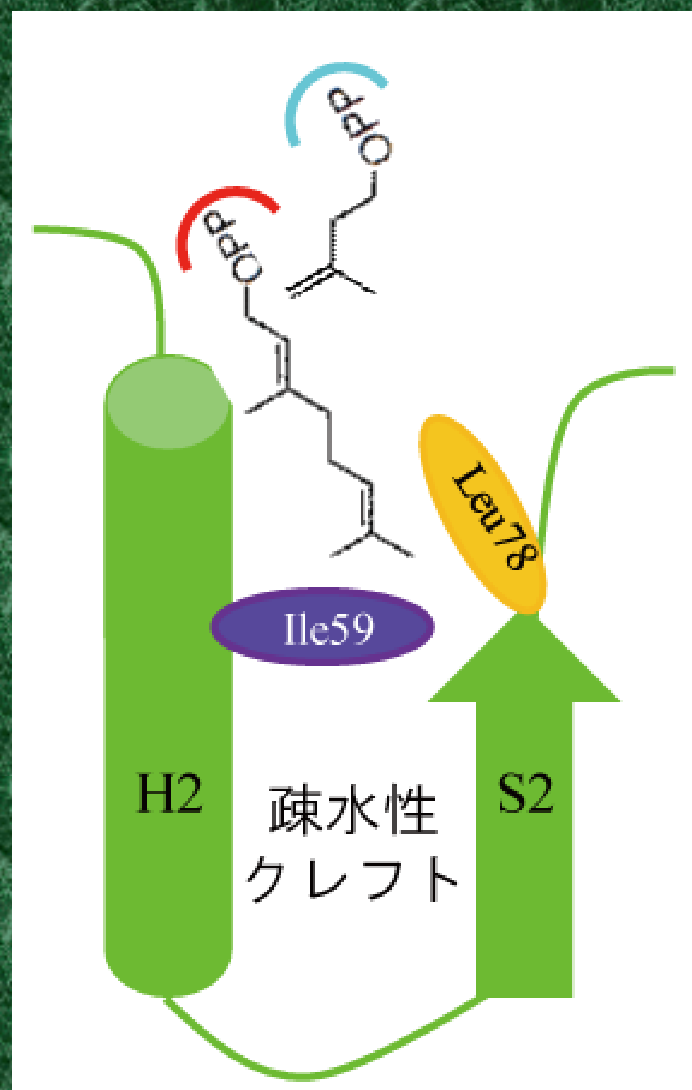


展開溶媒

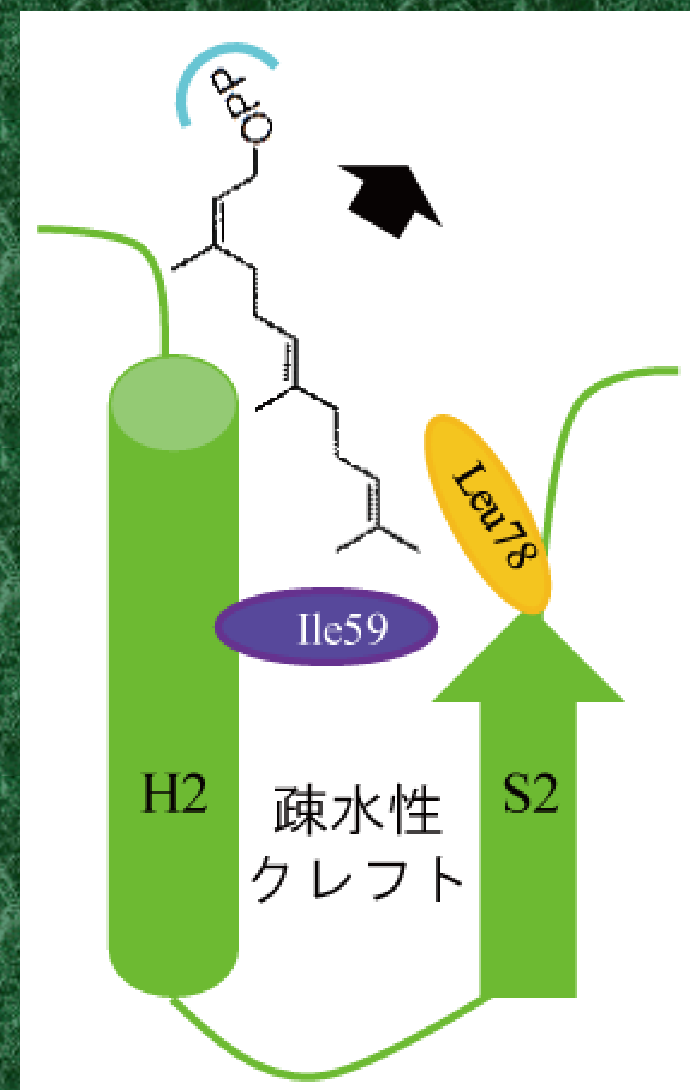
MeOH:Acetone=8:2







縮合反応

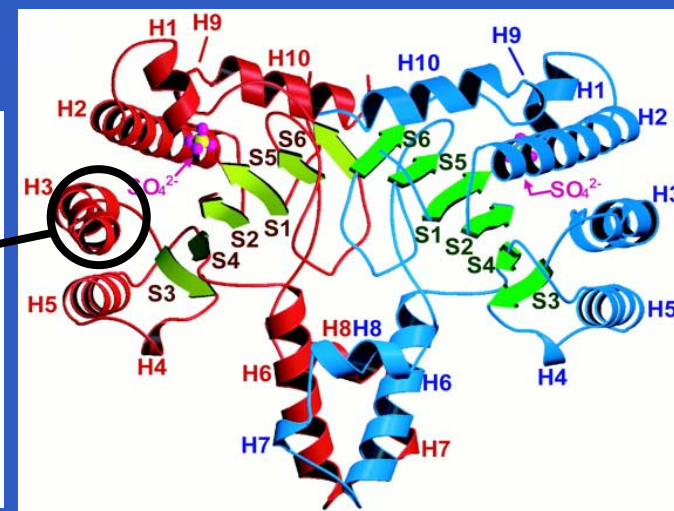




# Characteristic Region in Long-Chain *cis*-Prenyltransferase

Protein	Position	Sequence	Position	Sequence
Rv1086	100	L I E I I T D V V E E I C A	116	- - P A N
Rv2361	135	L M G F N R D V V R R R R D	153	- - T L K K L
<i>M. luteus</i>	88	L M K L P G D F L N T F L P	106	- - E L I E K
<i>E. coli</i>	85	L M E L F V W A L D S E V K	103	- - S L H R H
Rer2p	99	L M T L A R E R I R Q I T E R G E	120	- - L A C K Y
HDS	93	L M D L A R Q K F S R L M E E K E	114	- - K L Q K H
Srt1p	134	L M N L F T V K L D E F A K R A K D Y	167	- - K D P L Y

**Helix-3**



*M. Luteus* B-P 26 UPP synthase

Fujihashi *et al. Proc. Natl. Acad. Sci.* 4337-42 (2001)

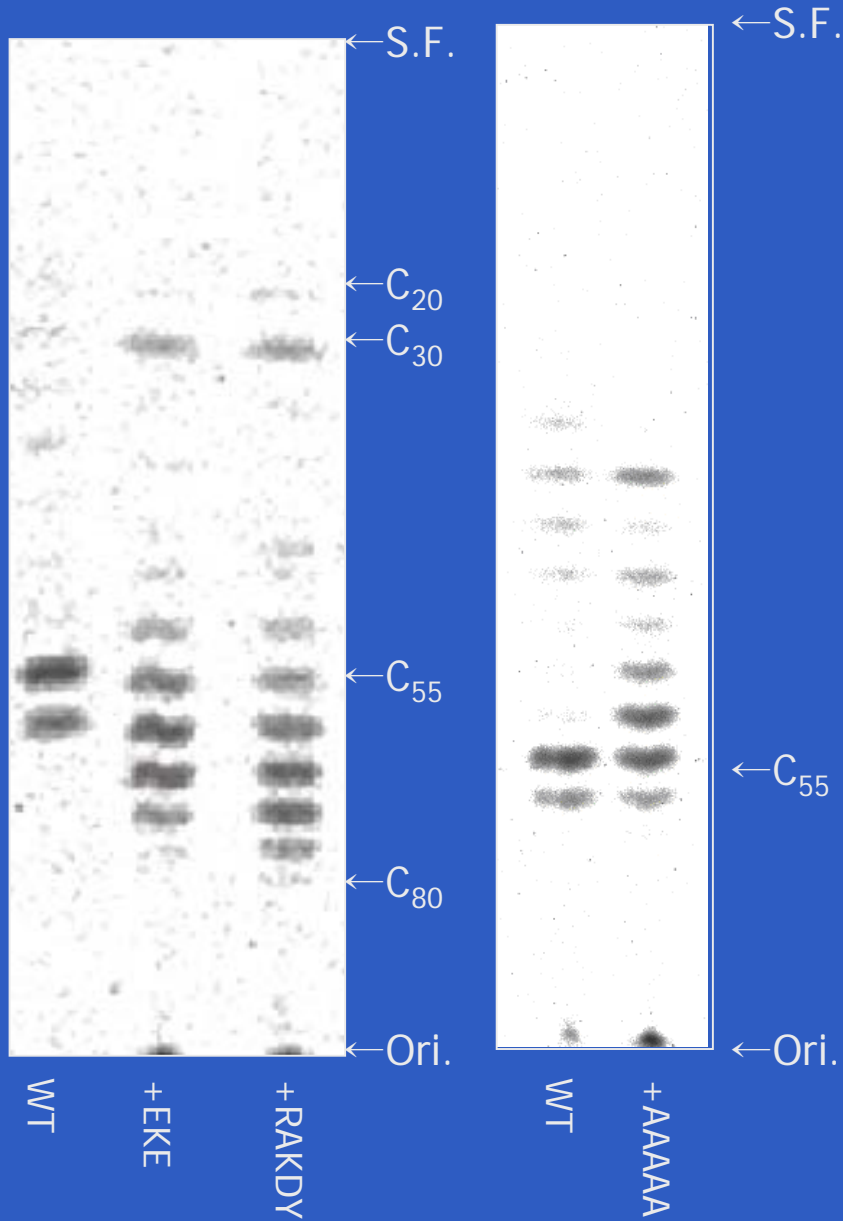
## *M. luteus* UPS

- |          |                      |          |
|----------|----------------------|----------|
| • UPS WT | 99- FLP              | ELI -104 |
| • +EKE   | 99- FLP <b>EKE</b>   | ELI -104 |
| • +RAKDY | 99- FLP <b>RAKDY</b> | ELI -104 |
| • +AAAAA | 99- FLP <b>AAAAA</b> | ELI -104 |

# Yeast Srt1p

- Srt1p WT 145- FAKRAKDYKDP -155
- Srt1p delta 145- FAK - - - - KDP -155

# Product Analysis of Mutant Enzymes



## ← *M. luteus* UPS mutant

Reversed phase TLC

solvent···Acetone/water(19:1)

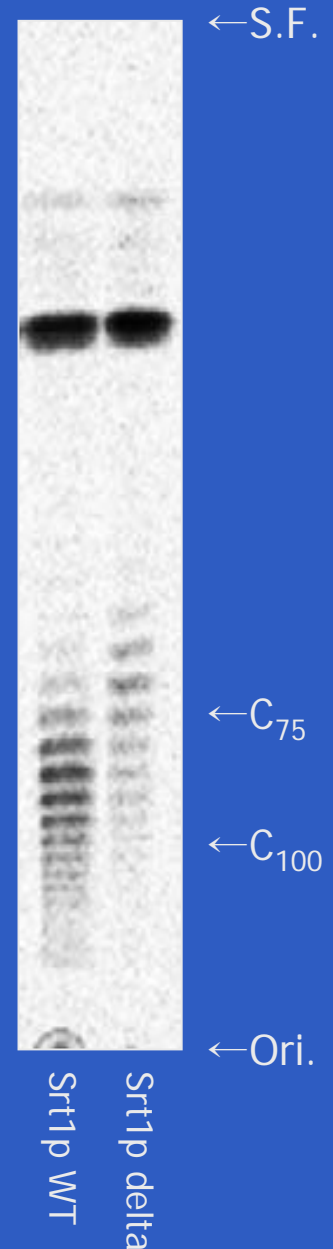
- UPS WT 99 FLP ELI 104
- +EKE 99 FLP **EKE** ELI 104
- +RAKDY 99 FLP **RAKDY** ELI 104
- +AAAAA 99 FLP **AAAAA** ELI 104

## Yeast Srt1p mutant→

Reversed phase TLC

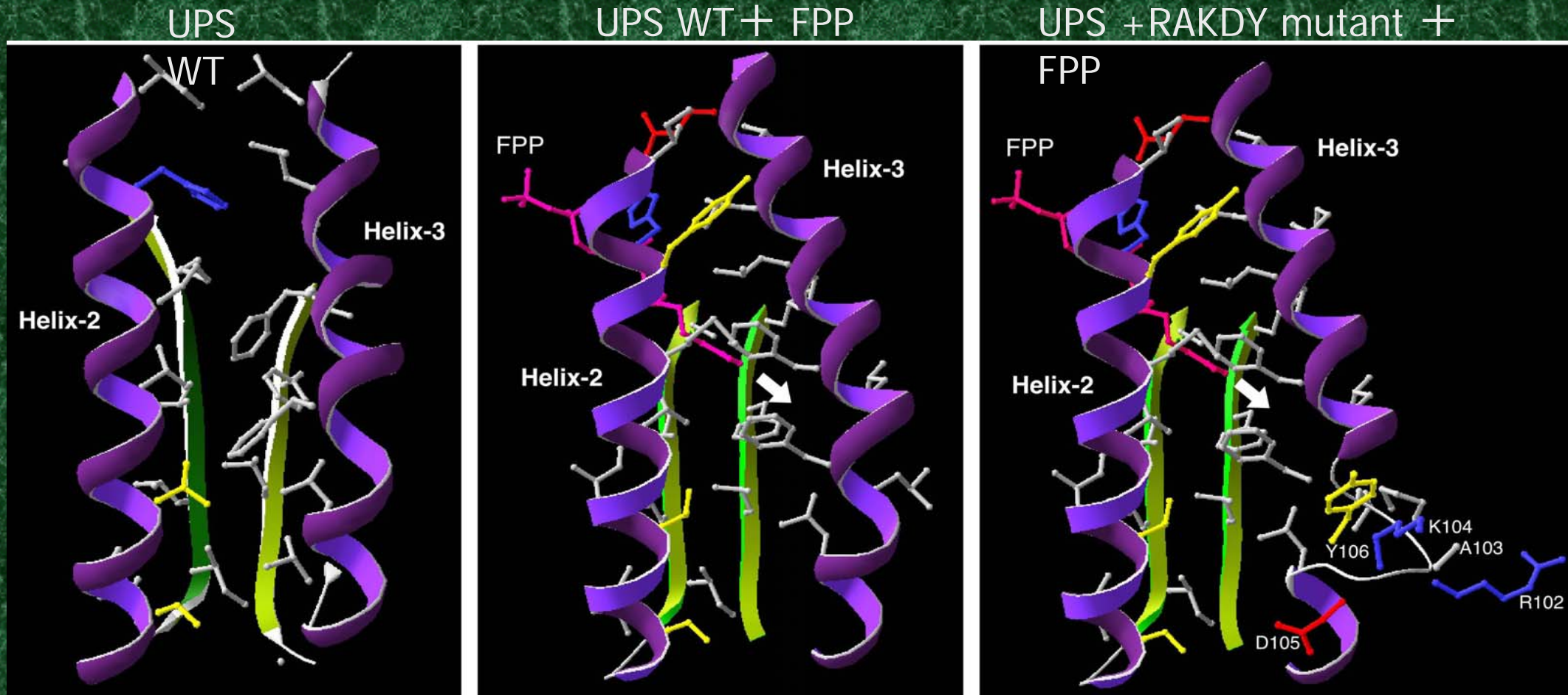
solvent···Acetone/water(39:1)

- Srt1p WT 145 FAK**RAKDY**KDP 155
- Srt1p delta 145 FAK - - - - KDP 155





# Structure model of the +RAKDY mutant



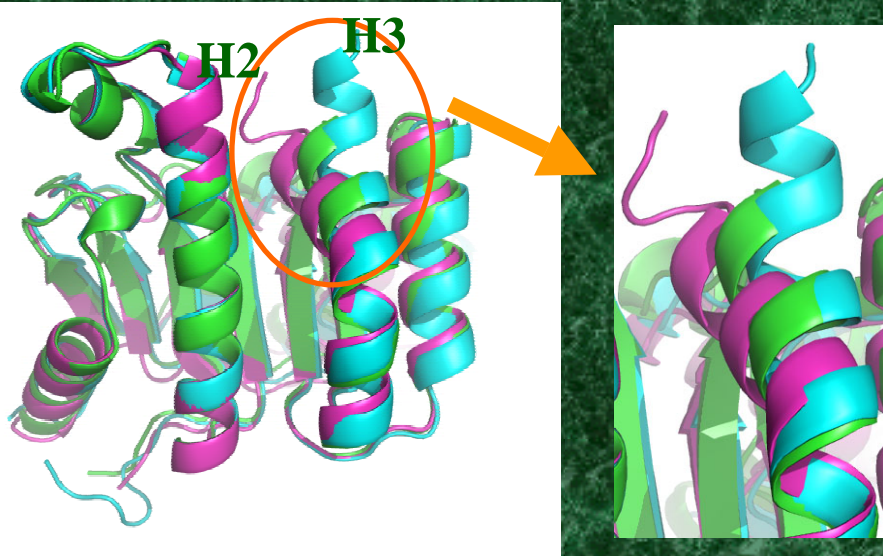
- Insertion of charged amino acid residues at Helix-3
  - • • side wall of large hydrophobic cleft
- ⇒ Insertion of charged residues may **control** the bending direction of the elongating product.



# Medium-chain typeにおけるHelix3の柔軟性

## UPS (Medium-chain type)

*M. luteus* B-P 26 UPS (Fujihashi *et al.* (2001))



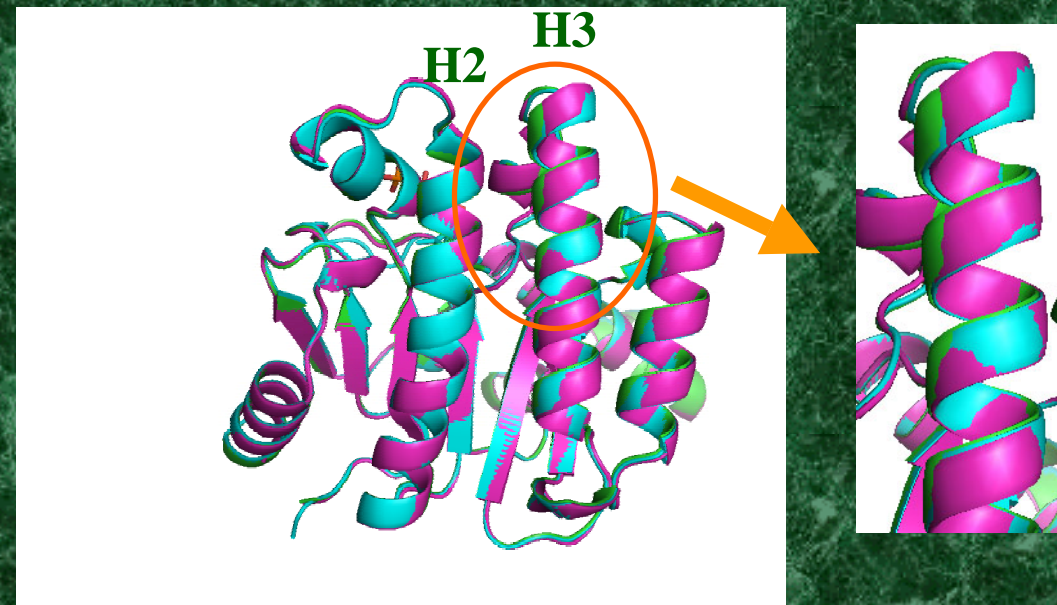
■ 1F75 (subunit A)

■ 1F75 (subunit B)

■ 福地さと子 修士論文 (2007)

## *cis*-FPS (Short-chain type)

*M. tuberculosis* (Wang *et al.* (2008))



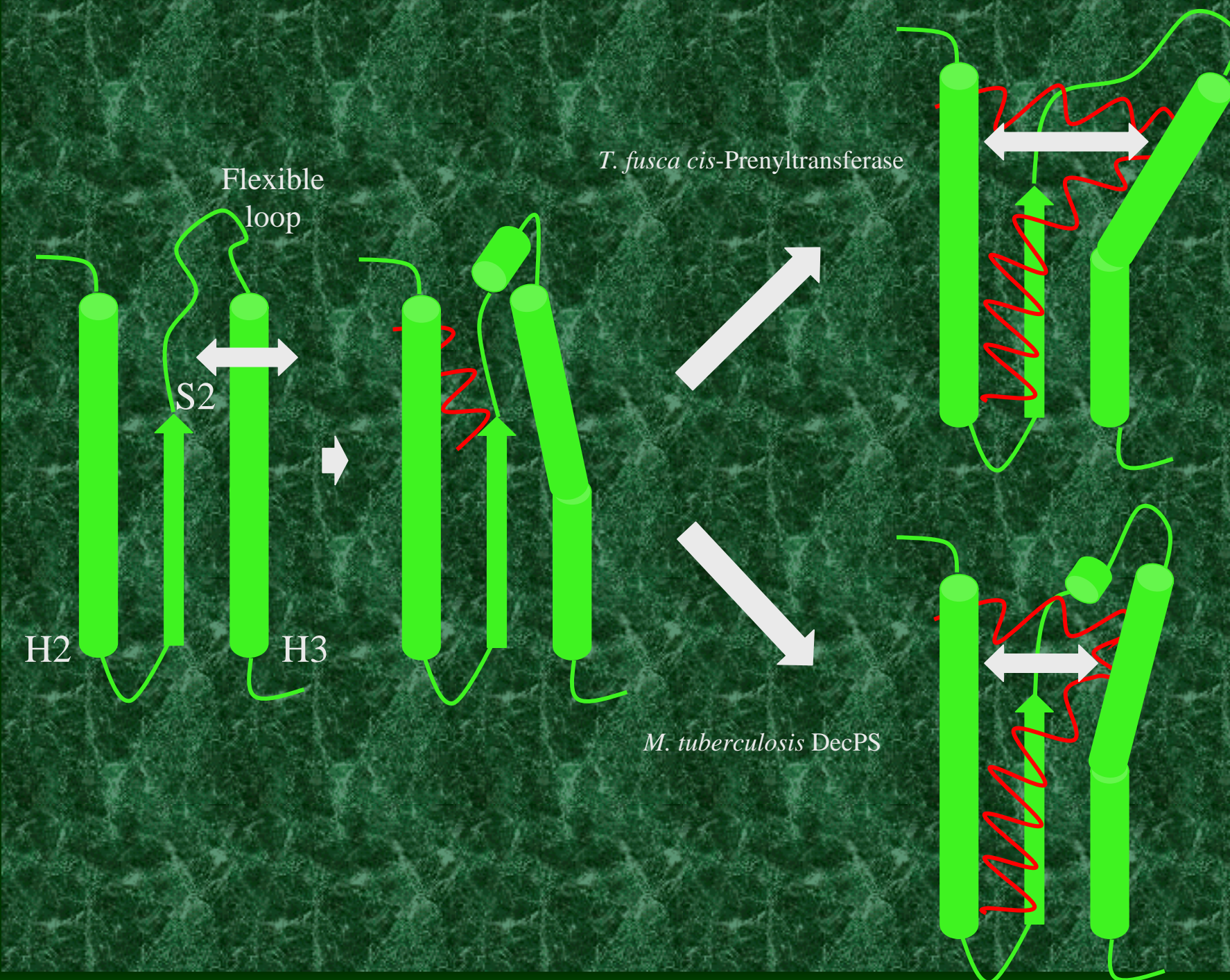
■ 2VFW: apo型

■ 2VG1: FPP結合型

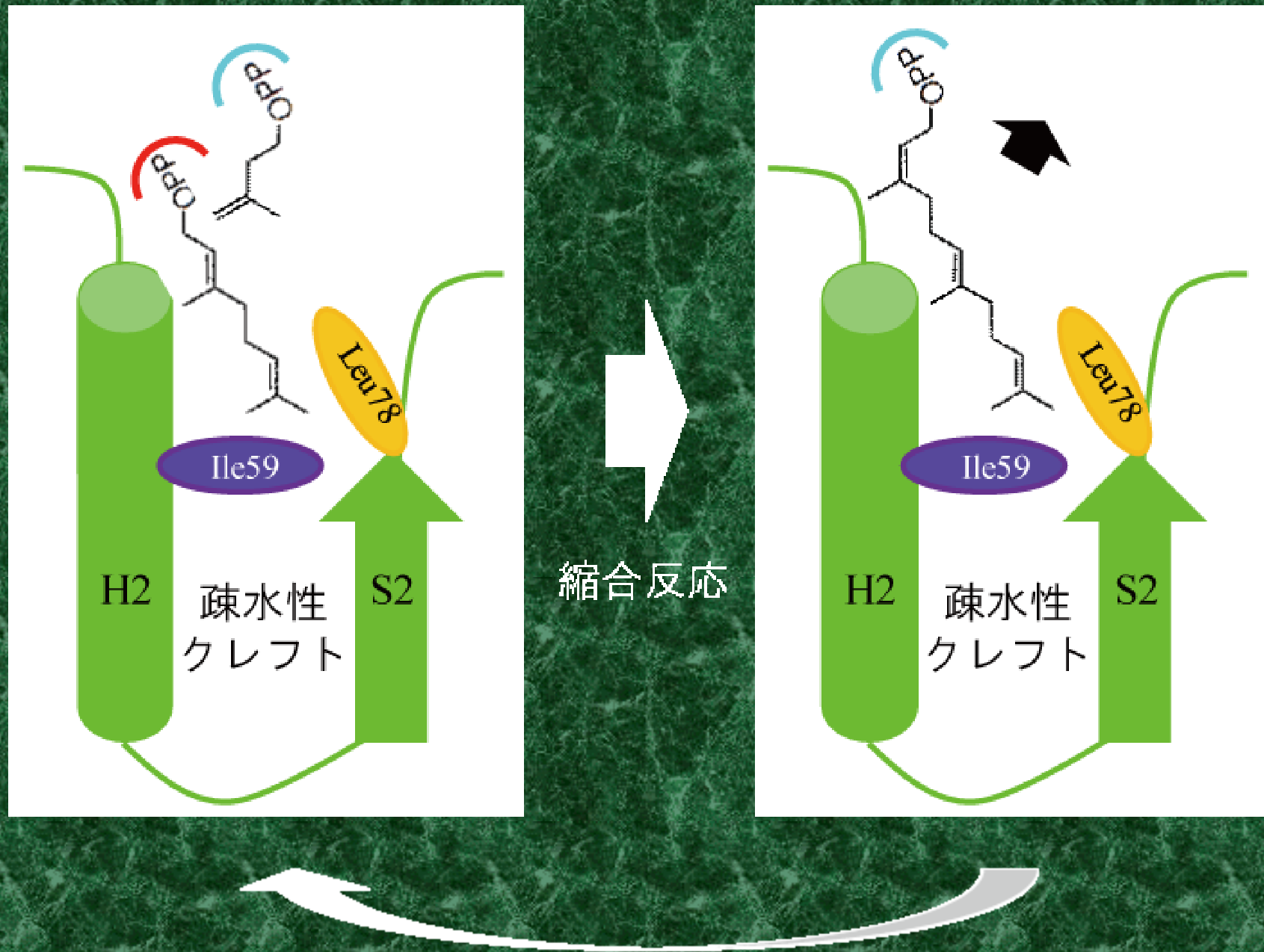
■ 2VG0: GPP analog結合型



# Medium-chain *cis*-Prenyltransferaseの鎖長制御機構



# Short-chain *cis*-Prenyltransferaseの鎖長制御機構





## 職員

佐上 博; Zhang Yuan-Wei; 高橋征司; 山下哲; 野池基義; 黒河博文

Xiao-Yuan Li; 小林 (三上) 真紀; 吉田 (矢後) 康子; 鹿野春子; 相原佳子

## 博士後期課程:

菊地 晃宏; 張 元偉; 清水 直人; 葛口 剛; 館山 誠司; 松村 欣宏; 小平 裕一

Yugesh Kharel; M. Navia Rani; 藤倉 慶太郎; 小関 弘恵知; 安保 貴永; 宮城 ゆき乃

## 博士前期課程:

臼井 聖尊; 境原 俊道; 金 達; 菅原 宏; 本田 敦子; 羽切 しのぶ; 萩原 隆

遠藤 章太; 高谷 昭行; 福楽 彩香; 武田 史世; 井上 明大; 大堀 裕治

三浦 洋之; 山田 恭之; 李 恵真; 寺内 大樹; 楠元 建; 城所 智広; 須藤 剛

高橋 武; 菊地 明香; 高山 大輔; 大木 彬乃; 関 俊輔; 大場 亮; 戸田 哲史

倉本 英次; 石川 乃梨子; 鈴木 康裕; 鈴木 俊秀; 相澤 陽介; 福地 (宇部) さと子

磯 達朗; 川井 秀之; 石黒 晃美; 菊地 崇剛

(1997-2009)

(1997-2009)

### **外国人研究生、リサーチフェロー**

Atiya Rattanapittayaporn; Kasem Asawatreratanakul  
S.Z. Moosavi-Nejad; Atiphon Phatthiya; Krueawan Yoonram  
Porntip Rojruthai; Paveena Nawakitkosol; Nopphakaew Chareonthiphakorn

### **共同研究者**

山形大学：榎雄二先生、大谷典正先生、弘前大学：長岐正彦先生、  
京都大学：三木邦夫先生、藤橋雅宏先生、東京農工大学：田中康之先生、  
トヨタ自動車(株)：小畑充生博士、竹下(小池)あゆみ博士  
Mahidol Univ.: Prof. D. Wititsuwannakul, Prof. J.Tangpakdee;  
Prince of Songkla Univ.: Prof. R. Wititsuwannakul; Univ. Utah: Prof. G.D. Prestwich



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